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Route to Novel Auxin: Auxin Chemical Space toward Biological Correlation Carriers

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1. Introduction

The search for links between chemical factors and physiological effects was already documented in the 17th century by William Harvey. He describes the blood circulation. Two additional hints of the transport of chemical substances were published later by Berthold in animals[1] and Darwin in plants.[2] Those issues were the bases of biological concepts for chemical messengers under the nascent terms organ-forming substances,[3] correlation carriers,[4] and hormones.[5–7] Further analysis reconsidered the active principles present in the tissue extracts capable of producing physiological effects when injected into animals as hormones.[8] However, for the active principles in the plant tissue extracts, the name used first was *auxin*.[9–12]

These chemical messengers, responsible for timing and regulation of growth and development as well as control of life transitions in animal and nonanimal alike, achieve their effects by (indirectly) altering gene activity. Moreover, plants and animals, rather than having a common biochemistry, only sharing points of contact[13] between parallel biochemical systems.[13–16] Plants are sessile, have cell wall and a photosynthetic apparatus but no nervous system. A single signal transduction system having the same or similar characteristics for both animal and plants is unlikely to be expected. Chemical messengers at tiny concentrations (hormones) in animals are amino acid derivatives, steroids, arachidonic acid derivatives, and the macromolecules peptides and proteins.[17] However, the best documented plant regulators (hormones) so far are generally small molecules with less than 100 atoms such as cytokinins, gibberellins, ethylene, brassinosteroids,[18] salicylates, jasmonates, and auxins.[19–21]

Chemical structural similarities connects most cytokinins as adenine derivatives,[22] brassinosteroids as steroid molecules,[23,24]

Noel Ferro, born in Havana, Cuba (1971), received his Master's degree from the University of Havana and Ph.D. degree from the University of Hanover (Leibniz Universität Hannover) under the direction of Professor H.-J. Jacobsen. He received fellowship awards from the German Foundation for International Development (DSE) in 2000 and the German Academic Exchange Service (DAAD) in 2003. His graduate research focused on the analysis of molecular mechanisms of plant hormones. He was a Research Visitor by Professor R. Carbó-Dorca and Professor P. Bultinck at Ghent University, Belgium (2004), and an Invited Professor in the Department for Applied Chemical Physics at the Universidad Autonoma de Madrid, Spain (2009). He was Assistant Professor at the Institute for Plant Genetics, University of Hanover, and currently has a research position at the Institute for Physical and Theoretical Chemistry, University of Bonn. Research interests are focused on structure and action mechanisms of biomolecules and crystals.

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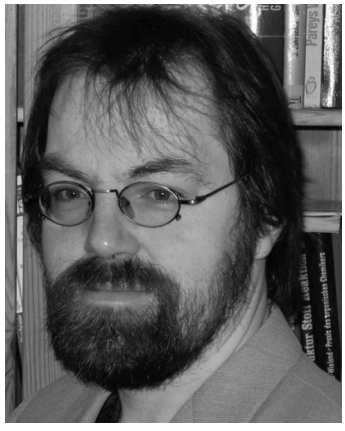
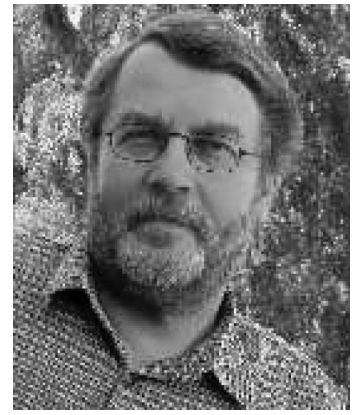
Thomas Bredow, born in Rinteln, Germany (1964), received his diploma (1990) and Ph.D. degrees (1993) from the University of Hannover. His doctoral study was on quantum-chemical calculations of photoreactions of transition-metal oxide nanoparticles under the direction of Professor Karl Jug. In 1998 he was an Alexander von Humboldt Feodor Lynen Fellow at the University of Milano-Bicocca, Italy, hosted by Professor Gianfranco Pacchioni. In 1999 he received a fellowship of the Australian Research Council and worked with Professor Andrea Gerson at the Ian Wark Research institute in Adelaide, South Australia. In 2002 he finished his Habilitation in Theoretical Chemistry at the University of Hannover. Since 2005 he has been Professor of Theoretical Chemistry at the University of Bonn. His current research interests include the development of theoretical methods for the description of condensed matter and large organic molecules, heterogeneous catalysis, and defect formation and mobility in ionic solids.



Thomas Reinard, born in Leverkusen, Germany (1963), graduated in 1988 from the University of Bonn and received his Ph.D. degree from the same university, working under the supervision of Professor H.-J. Jacobsen on auxin binding protein research in 1992. Now he is working as a lecturer at the Leibniz University of Hannover at the Institute of Plant Genetics and is Head of the Biochemistry group. Besides auxin research, his secondary research interests deal with the production of recombinant antibodies and other pharmaceutical proteins plants.



Hans-Jörg Jacobsen, born in Wilhelmshaven, Germany (1949), received his diploma in Biology (1974) and Ph.D. degree (1978) from the University of Bonn. His doctoral study was on auxin effects on RNAse-isozymes under the direction of Professor Hermann P. Müller. He worked as a postdoctoral fellow in Brasilia (1980/81) at CENA in Piracicaba and collaborated with Professor Kees Libbenga (Leiden, The Netherlands) on auxin binding proteins. In 1985 he finished his Habilitation in Genetics at the University of Bonn. Since 1991 he has been Professor for Molecular Genetics at the University of Hannover (now Leibniz Universität Hannover). His current research interests are in the field of developing trangenetic systems for improving crop plants to better resist pests and diseases as well as abiotic stresses. He has a continuing interest in getting the auxin enigmas solved.

abscisic acid derivatives as terpene,[25] ethylene derivatives as simple alkenes[26] and most gibberellins share the ent-gibberellane skeleton,[27] as well as the salts and esters of salicylic acid and jasmonic acid are known as salicylates jasmonates respectively.

In contrast, a quite diverse group of chemicals represents the auxins. Few naturally occurring (indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), phenylacetic acid, and some of their chlorinated derivatives) and many synthetic molecules (e.g., phenoxyacetic, naphthalene acetic acid derivatives) were tested under the name "auxin" without further biological evidence.[28–31]

The IAA, the main natural auxin's representation, cannot be enclosed in the plant kingdom. It was first identified in mammals and is currently (its derivatives as well) used in treatments against asthma and chronic obstructive pulmonary disease (COPD)[32,33] as well as in clinical tests as anticancer drug.[34] Urinary determination of IAA is clinically significant as a tumor marker in the diagnosis of malignant diseases.[35,36] It was also found that IAA transmits effects among plants and bacteria cells and therefore can have a direct effect on bacterial physiology.[37,38]

IAA's functions, as a multivalent signaling plant molecule, must not and cannot be restricted to a hormone-like action. Further contributions as first messengers would be the morphogen- or neurotransmitter-like action. A morphogen provides spatial information by forming a concentration gradient that subdivides a field of cells, which is tightly connected to the action of auxin transporters as the primary cause for the formation of local gradients.[39] The neurotransmitters provide many fascinating structural similarities to IAA. It is currently investigated to understand more about the role of neurological compounds in the inner mechanism of plant metabolism, plant environment interactions, and the impact of plant substances on human neurology.[40]

The so-called auxin signal complex in plants involves more than one thousand molecular effectors, several auxin binding proteins (sometimes called "receptors"), as well as many biological effects. To date any proposed hypotheses about the structure−activity relationships in auxins have been refused or accepted consistently with respect to the biological surrounding. The traditional deterministic viewpoint of a ligand to act on an individual target based on the lock−key, hormone, and receptor theories has been facing considerable challenges to explain the fundamental problems and peculiarities of auxin effectors as correlation carriers[4,12] in plants.

The term correlation carrier has connections to the core conception of the chemical messengers. Starling's work "The chemical correlation of the functions of the body"[5,6,41,42] has been mostly misinterpreted in the biological context as functional substance−activity dependence. His approach "the activity and growth of different organs... are determined and

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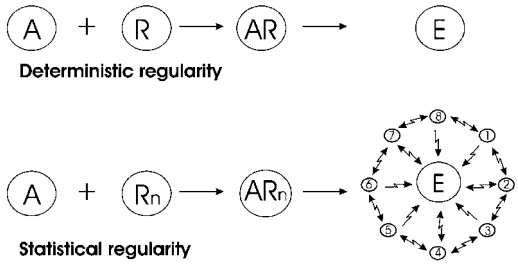

Figure 1. Schematic representation of deterministic and statistical regularities focus on the auxin-like effect. Deterministic regularity (top) represents the model to explain the response by molecular interaction kinetics.[88] Statistical regularity (bottom) tries to describe the complexity of relationships between binding and actions in a biological network where the molecule has a prospect of causal participation on the effect. A is an auxin molecule; R is a receptor. Rn is a receptor that is a component of a network. AR and ARn represent the complex and E the effects. The last term of the statistical regularity is the network of coordination to induce one of the probable effects. Therefore, numbers 1−8 represent different propagation of the effects[8] of receptor occupation (ARn) that can contribute to a biological effect (E). The arrow tips indicate the directions of the relations. An arrow with one tip indicates a unidirectional communication. An arrow with two tips indicates either a bidirectional communication or a unidirectional communication in both directions. The zig-zag arrow means that the processes can have several twists and turns or will not be always present. All depend on a background of genetic information and physiological environment.

coordinated among each other by chemical substances" was focused on relation per se or interdependence of effects (correlation), consequently, placing the chemical factors as correlation carriers, which within a reasonable space of time will control the physiological products. He looked forward[43] to current concepts such as signal transduction pathways, dynamic genome,[44] and a dynamic approach of the chemical forces of the signal substances.[45]

Following this scope, the scientific approach to the auxin phenomena so far has been (1) underestimating the influence of weaker molecular interactions than the hydrogen bond. Such interactions, like van der Waals (VdW), have been demonstrated to induce "promiscuity of chemical recognition" (see the presence of three different auxin molecules in the TIR1 binding site[46]) coming from the ambiguous recognition of the electronic bulk of different rings in the receptor and (2) lacking of analysis of the statistical regularities pointing to optimizing multiple structure−function relationships in the biological organism. Both views change the coordination of biochemical and physiological functions among cells and organs (Figure 1). F1

This review summarizes the present state of knowledge on structure−activity relationships of auxin-like molecules and their biological repercussion based on the following objectives: (i) analysis of the auxin concept and its evolution; (ii) discussion of the available structure−activity theories; (iii) auxin diversity and pleiotropic effects; (iv) more than one set of structural requirements for auxin-like activities and its implication on the receptor(s) evolution; (v) fraction of quantum similarities measured as a chemical molecular property with predicted capability of the biological influence; and (vi) classification of auxin molecules based on the domain of molecular similarity with a significant influence on the biological activity.

Alternative ways to understand and focus on the mechanism of action of the most often applied substances in plant physiology and agriculture (as herbicides, plant growth regulators, etc.) are presented.

2. Contextualization of the Auxin Phenomena

The term "auxin" is simply derived from the Greek word "auxein" (= to increase, to help). It was introduced after the isolation of the compound auxin-a by Kögl and Haagen-Smit in 1931.[9] However, the proposals "auxin-a" and "auxin-b" did not clarify the existence of a signal molecule as the cause of the auxin phenomena. Their structural detection can now be considered as a scientific miracle since the scientific scenario from the 1930s did not allow the elucidation of such structures.[47]

The auxin as a molecular entity is still unclear and covers a broad range of chemical structures. In the 1980s even the brassinosteroids and fusicoccin were allocated as auxin molecules in accordance to their activity[48,49] (Figure 2). F2 Nowadays, brassinosteroids are considered as a new class of highly active plant growth promotors[18] and fusicoccin is regarded as a fungal toxin.[50]

2.1. Auxin as Molecular Entity

The onset of the first phase (auxin chemical concept) (Figure 3) of evolving a concept for auxin activities driven F3 by molecular structure was described between the 1930s and the 1970s.[11,28,30,51] In 1935 Went wrote "the physiological name *growth substance* and the chemical name *auxin* are interchangeable...".[10] Ten years later Went wrote "chemical isolation and identification of indoleacetic acid from vascular plants has been accomplished. This makes it necessary to use the term *auxin* as a generic name for all substances, produced by plant as growth hormones or as correlation carriers, which gives response in the Avena test".[12]

Went restricted auxin activities to physiological effects (cell elongation) coming from one plant genotype (Avena test) and the term "auxin" to a chemical entity with partial or without scientific evidence to prove it. IAA was firmly established as a natural auxin in higher plant tissues only in the 1970s when its structure was conclusively identified in *Picea, Pinus*, and more than 18 angiosperms.[52]

This procedure on auxin research has generated different speculations about the auxin concept to date: (1) Auxins are organic compounds, which promote growth (irreversible increase in volume) along the longitudinal axes, when applied in low concentrations to shoots of plants.[53] (2) Auxins is the generic name for a group of substances resembling the endogenous auxin molecule IAA in action or in structure and can be divided into several classes: the indol compounds, the phenoxyacids compounds, the benzoic acid compounds...[54] (3) Auxins are compounds that cause cell enlargement of plant cells.[55] (4) An auxin is a compound that has a spectrum of biological activities similar to but not necessarily identical with those of IAA. This includes the ability to induce cell elongation in coleoptile or stem sections, cell division in callus tissue join to cytokinin, promote root formation at the cut surface...[56]

Thus, we are confronted with a dual task. (i) Clarify the molecular population ground states of the auxin signal molecules to focus on the action mechanism. (ii) Define the molecular acceptor(s) in the biological environment. The main objective of this review is the first point; however, we will consider the biological context to obtain details about the problem going on.

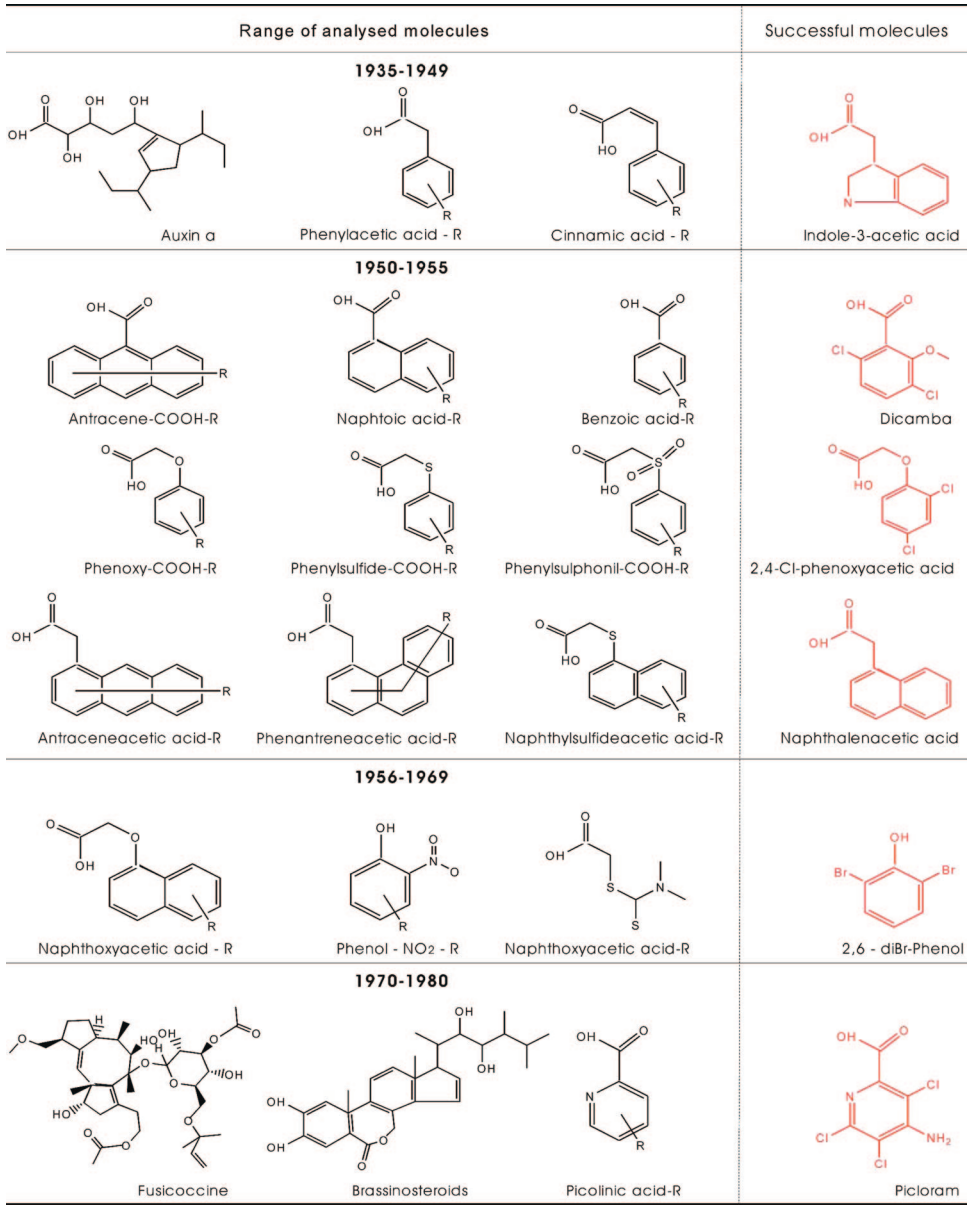

Figure 2. Chronological schematic representation of the auxin-like molecules. The actually successful compounds are in the right panel. 1930−1949: the first compounds assumed to be responsible for auxin activity were isolated and some of them analyzed experimentally. The 1950s were the very active decade to learn about details regarding the analysis of different compounds. At the end of 1950s and in the 1960s new compounds were found like dithiocarbamate (without ring) and phenol (without side chain) derivatives. Derivatives of picolinic acid like picloram were found to be very usable in practical applications such as herbicides. Derivative compounds successfully known as active auxins and more exploited as plant growth regulators have been shown. In the 1970s and 1980s fusicoccin and brassinosteroids were considered auxins by some authors.[48,49] The letter "R" in the scheme represents the different substitutions (F, Cl, Br, I, OH, CH_3, NO_2,...) in the ring system of different molecules.

2.2. Auxin Perception, Signal Transduction, and Gene Expression

One of the goals of any structure−activity analysis is to predict characteristics of the biological acceptor. At present, the classical hormone concept underlying the auxin structure−activity rules ("*auxins act as a kind of co-enzyme or ergon at the growth centre, which is a protein or enzyme surface of highly specific shape*"[57]) is obsolete as it is not consistent with experimental data anymore. The present section does not go in details about the mechanisms of absorption and distribution (Pharmacokinetics). It just exposes the complexity of interactions at the pharmacodynamic level, which affect the work strategies for structure−function analysis.

2.2.1. Complexity of the Auxin Reception

A second research phase (auxin biological concept) in auxins began in the 1970s with the proposal of the so-called first receptor candidate auxin binding protein 1 (ABP1)[58] (Figure 3). It was assumed as the target molecule or auxin receptor, and the next edition of the *Encyclopaedia of Plant Physiology* in the 1970s did not dedicate any chapter to the analysis of structure−activity. All efforts were focused on the action mechanisms of signal transduction for auxins under the concept of hormone action in animals. Many research activities were carried out on the following topics: auxin perception, transport machinery, transport routes, and interactions with other hormones (for recent reviews see, e.g., refs 59–61). However, the successes of describing some

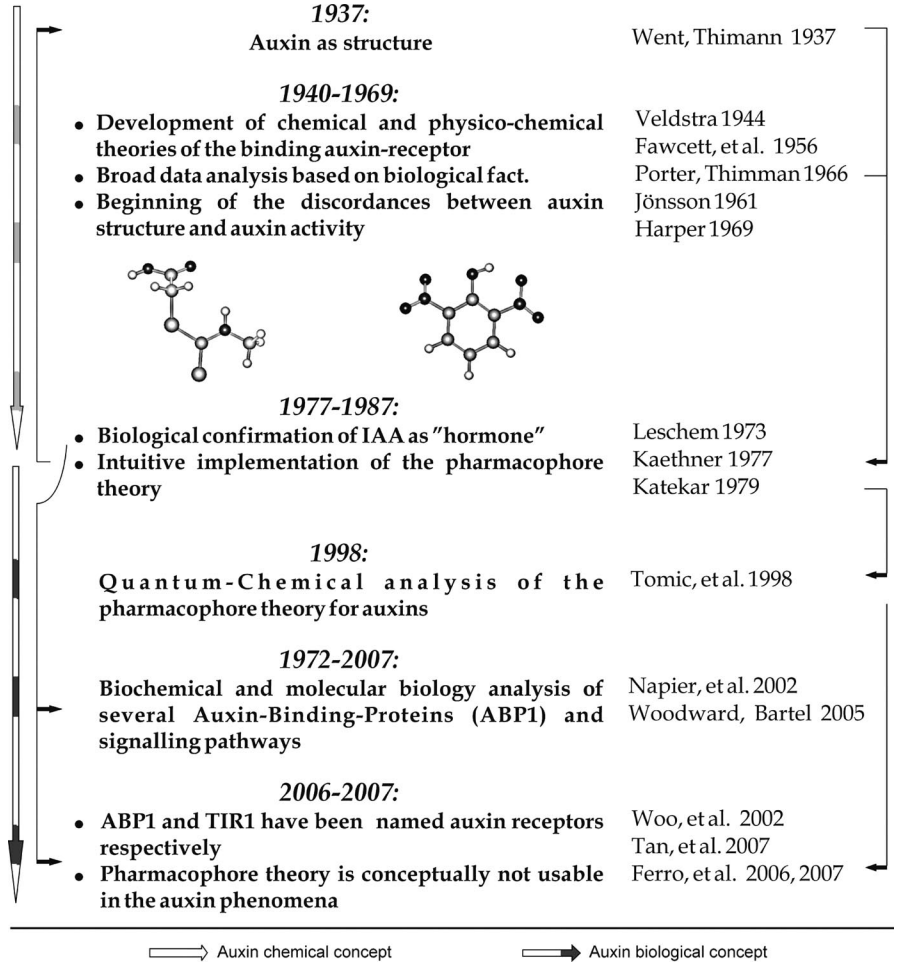

Figure 3. Chronological evolution of the auxin concept. Two different periods (arrows at the left) of the auxin concept are shown. The first is dominates for the chemical determination of molecules and properties engaged with such kind of biological responses. A second period, biologically significant, began with the first requisite to be a phytohormone, confirmation of IAA in most of the plants. Actually, two different auxin-binding proteins have been proposed as receptors with crystal analysis of the binding "protein−auxin" ABP1[160] and TIR1.[46]

animal and bacteria receptors have not been matched to the same extent for plants. Plant hormone receptors have proven to be elusive research targets, and the role of ABP1 in plant physiological effects is still under debate today.[62]

The transport inhibitor response 1 (TIR1, part of the protein complex Skp1-Cullin-F-box (SCF^{TIR1})) was recently described as a second putative auxin receptor. Two differences with respect to the allosteric regulation disclosed a new biochemical mechanism of the interaction "protein−small molecule−protein" in SCF^{TIR1}: (a) the auxin activator is working as a linker molecule between both proteins within the binding site and (b) the expected atomic rearrangement of the protein is not observed. An explanation of the action mechanism of the proteinic complex (SCF^{TIR1}) requires very accurate and precise methods from experimental and theoretical viewpoints.[46] TIR1 binds auxin-like molecules with potential effects on activity.[63,64] However, there is not sufficient biological information to confirm its physiological impact on short-term physiological effects of auxin such as proton pumping,[65] wall loosing,[66,67] and other mechanisms of fast responses.[68] TIR1 is not supposed to be responsible for the whole context of auxin activity. It cannot be assumed as the only auxin receptor.

Thus far, the expectation of a bona fide receptor system proposed by Venis[69,70] does not say much in favor of both ABP1 and TIR1 binding proteins as "auxin receptors". Some criteria are not fulfilled by the auxin binding proteins[70] and auxins as phytohormones[69] but for the promiscuity of interactions:

(i) Binding specificity for different hormones analogues should be approximately in accordance with the relative biological activities of the compounds. First, the physiologically inactive (with respect to IAA)[64,71–80] 2-NAA (CAS 581-96-4) shows the best binding affinity to ABP1.[81] It is necessary to clarify that a high binding affinity means that 2-NAA is active in the biological environment and its antiauxin activity is most probably by competition.[82] Second, mutations in a different TIR1 gene family member in *Arabidopsis* displayed resistance that was highly selective for a novel picolinate auxin but not 2,4-D or IAA.[83] It indicates that preferences for auxins in terms of biological responses may be determined by the selectivity of different members of the TIR1 protein family or could open the way to selectivity of different auxin members by different families of proteins.

(ii) Binding should lead to a hormone-specific, biological response. First, it is known that 1-NAA (CAS 86-87-3) and IAA bind to nonplant-protein bovine-like serum albumin (BSA) and Human Kynurenine Aminotransferase,[84] respectively. BSA and ABP1 com-

parisons of the affinity for auxin, antiauxins, and other structurally related competitors are known.[85] Second, auxin-like molecules show mainly pleiotropic effects.[15] An ABP1-independent pathway was described recently, which is much more sensitive to IAA than the ABP1-dependent one.[86] TIR1's binding site recognizing diverse auxin structures IAA, 1-NAA, and 2,4-D (CAS 94-75-7); this result was quite unexpected.[46]

(iii) Binding should be limited to hormone-responsive tissue. It is difficult to define for plants as most tissues are responding to hormones. It is still being one of the most discussed issues in phytohormone research since the 1980s.[15,87,88]

Besides both putative receptors commented on above, different extracellular and intracellular auxin binding sites are involved in the perception of auxin molecules.[61,89,90] Consequently, many auxin binding proteins have been characterized, several without physiological significance.[58,91] In addition, two different families of auxin response factors (ARF) are required for controlling the expression of auxin response genes: auxin response elements and AUX/IAA repressors.[92]

Further dynamic consequences are the signaling pathways in higher plants. Their genes fusion and fission addresses several critical points with respect to cross talk, signal integration, and specificity.[93] G-protein subunits perhaps trigger a multisignal of the cell cycle affected by auxin and other hormones.[94] In addition, ubiquitination-inducing hormone receptors are playing important roles in the perceptions of auxin and jasmonate derivatives.[46,95,96]

2.2.2. Dynamics of Auxin Signaling

Further enigmas of auxin signal perception have been observed. (a) The influence of pH on the ionization and biological activity of auxins: (i) the binding activity of the ABP1 exhibits optimal results at pH 5.5, which indicates that it occurs extracellularly. However, only 2% of this protein has been found outside of the cell membrane (approximately 1000 molecules per cell, which seems to be sufficient to induce signal). It remains unclear why 98% of the same protein sticks in the endoplasmatic reticulum and does not bind due to inaccessibility and/or pH $\approx$ 7. (ii) The dissociation of the carboxyl group in the intracellular space (pH $\approx$ 7) of 2,4-D has been used to explain accumulation as a toxic ion ($R\text{-}COO^-$),[97] while extracellular (pH $\approx$ 5) 2,4-D exists in the lipophilic form (RCOOH). This implies the influence of the dissociation constant of the different auxin molecules in the transport through membranes. However, phenol compounds tend to increase the stability of the deprotonated form.[98] They have toxicity at a similar concentration as the classical auxins and auxin-like activity at higher active concentrations.[79] (b) Long-term effects like morphogenesis or gene expression profiles and fast auxin effects (which usually occur within minutes) use different modes of action (possible unravelled signal transduction cascade). Classically, the best way to provide evidence about a receptor function is finding a lethal mutation[99,100] as maximal genetic control of the receptor functions. Therefore, as long as any auxin lethal function is found, the discussion about mediators of auxin action in plants is open but not precisely about receptors. The situation resembles more a functional network of intermolecular interactions.[101] (c) Auxin transporters are another important group of auxin interaction proteins. Membrane proteins like plant-specific pin-formed (PIN) proteins[102] are convincingly involved in auxin influx and efflux[60,103–107] as well as in endocitosis phenomena.[39] Other findings about auxin transporters provide evidence for the occurrence of transport through ATP-binding cassette (ABC) proteins, indicating a complex array of primary and secondary active transport processes involved in auxin distribution.[108,109] ABCB19 has been found as IAA transporter with in the root (acropetal), while PIN1 is accomplishing shoot transport (basipetal).[77,110,111] Moreover, the quantitative nature of the interacction AUX1 with IAA as part of the AUX/LAX family of auxin importers[112] open a new manner to select the auxin binding ligands. A further integrative picture of auxin transport could be the focus on resent advances on effects of in-channel interactions and blocking[113] and the occurrence of PIN proteins in the ER.[114]

In summary, the term "auxin activity" has become a quite enigmatic biological phenomenon characterized by the incompatibility between the intuitive hypothesis of chemical requirements for auxin-like molecules and the biological facts at the pharmacological, biochemical, and molecular biology level. Careful discussion of the presently available facts above suggests that auxins act via modulation of multiple proteins rather than the dominant paradigm to act on individual targets. Therefore, we should validate target combinations and optimize multiple structure−activity relationships.[101,115] This necessitates characterizing the chemical moieties of auxin-like molecules more deeply by using tools of theoretical and combinatorial chemistry.

2.3. Structure−activity Theories and Limitations

The main objective of structure−activity analysis is to identify the structural characteristics which induce a function(s). The next step is to predict the family of active compounds. Consequently, the first relevant structural rules for auxins (from a chemical point of view) were formulated at the end of 1930s.[11,28] They stated an auxin molecule requires (i) a ring system as a nucleus, (ii) at least one double bond in the ring system, (iii) a side chain containing a carboxyl group with at least one atom removed from the ring, and (iv) a particular space relationship between the carboxyl group and the ring. However, no physiological impact of these minimal requirements was postulated. The rules are incompatible with active naphthoic and benzoic acids as well as phenol derivatives, described later as auxins (Table 1).[30,79,116] Other structural requirements for auxin molecules were considered between the 1950s and the 1970s. T1

2.3.1. Chemical Approaches

The theory of Hansch and Muir[117,118] is related with the ortho-effect phenomenon or the two-point attachment theory (TwoPA) (Figure 4). This theory predicted that bond formation between the active site of a protein and an aromatic ring should occur essentially by a chloride substituent in the ortho position. Muir et al. assumed that the position of attachment on the ring would depend on the particular combination of steric and electronic factors.[119] Other analyses regarding the TwoPA theory concluded that hydrophilic substituents (OH, NH_2) do not confer activity of the resulting derivates but only lipophilic ones (Cl, Br, I, CH_3).[120] A chemical attachment system in hormones implies that a physiological response follows only by means of a reversible fixation to the receptor. However, the covalent binding F4

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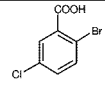
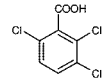
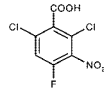
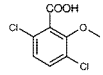
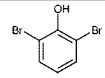
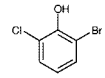
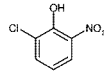
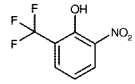
Table 1. Active Benzoic and Phenol Derivatives

Name	Structure
Benzoic acids	
2-Br-5Cl [CAS: 21739-93-5]	
2,3,6-triCl (Trysben) [CAS: 50-31-7]	
2,6-diCl-4-F-3-NO_2	
Dicamba [CAS: 1918-00-9]	
Phenol	
2,6-Br [CAS: 608-33-3]	
2-Cl, 6-Br [Harper, 1969[	
2-Cl, 6-NO_2 [Harper, 1969]	
2-CF_3-6-NO_2 [Harper, 1969]	

postulated in this theory can not explain a reversible enzymatic process.

The second chemical approach was postulated by Thimann[121] as separation charge theory (SCT). Its only molecular requisite for activity has become the most popular theory. An intramolecular distance of 5.5 Å between the positive and the negative atoms is accepted in text books and journals even in the 21st century.[122,123] However, Thimann himself described the low activity of 5,7-dichloroindole-3-acetic acid as a serious unsolved deviation.[31,51] Jönnson, who analyzed the structure−activity relationship of more than 600 auxin molecules, rejected the SCT.[30]

Further analysis using quantum chemical self-consistent field molecular orbital calculations did not support certain details of SCT. They rather show a net negative charge on the position (atom) regarded as carrying a positive charge

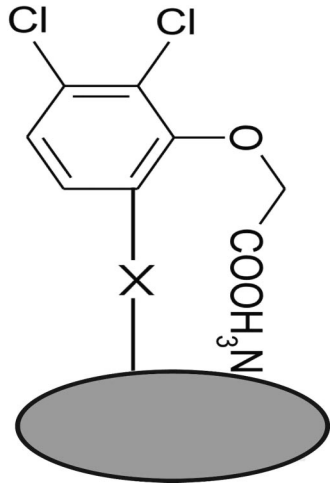


Figure 4. Two-point attachment theory.[117,118] Speculative irreversible chemical bond at the protein binding site.

for both IAA and 2,4-D.[124,125] Recent quantum-chemical calculations at the ab initio level[126] confirm that the position of the N in the pyrrole ring (initially proposed as a positive atom[31]) makes the indole more aromatic than its isomers, while a substituent at position 3 does not significantly change the aromaticity properties of the indole system. Our current quantum chemical calculations also do not confirm the SCT (Figure 5). In addition, one of the most active auxin molecules, IBA (CAS 133-32-4), has a separation of about 7 Å. F5

2.3.2. Physico-Chemical Approaches

Veldstra suggested that the action of an auxin consists in a physico-chemical influence of a boundary (PCIB). Two structural features are required: (i) a basal ring system with a high surface activity and (ii) a carboxyl group in a definite spatial position with respect to the ring system (Figure 6). F6 He postulated that the function of the plant growth substance consists in the physico-chemical influence of the boundary, the nature of the ring system determining the degree of adsorption of the active molecule to the boundary, and the physiological function properly being attributed to the carboxyl group.[120,127] Later, due to the measured increase of activity by chlorination of the phenoxyacetic acids, he postulated that a high surface activity in the ring system was not sufficient for the auxin action. A certain balance between the lipophilic and the hydrophilic part of the molecule was assumed to be essential.[30,128]

On the one hand, this theory, controversially, abandoned the idea of a molecule as one system focusing on the carboxyl group as a region biologically responsible while the ring system is in charge to disturb the activity. Veldstra[120] concluded that hydrophilic substituents like NH_2 do not confer activity of the resulting derivates. Nevertheless, Picloram is a well-recognized auxin derivative which indeed has a NH_2 group in its structure. On the other side, Veldstra assumed that the auxin molecule is not bound by strong chemical interactions at the site of action but is loosely and reversibly attached by many weak bonds (hydrogen bridges, electrostatic attractions, VdW forces).[120]

The three-point attachment theory (ThreePA) attempts to explain certain phenomena, which were found to be inconsistent with Velstrad's theory. ThreePA considers a broader range of substances: (i) a flat ring system, (ii) a hydrogen atom close to the carboxyl group, (iii) a special configuration of the side chain with respect to the ring, and (iv) the free rotation of the side chain at the bond joining to the ring seems to be structurally required for its activity (Figure 7).[29,129,130] F7

The use of the SCT as requisite for the auxin activity is avoided. The mechanism suggested two hydrophobic areas localized in the receptor, either of which could complement the aromatic ring systems, and a single positively charged site to accommodate the carboxylate group.[131] Unfortunately, this theory could not explain the activity of benzoic acids and phenol compounds, and the free rotation of the side chain is not a prerequisite for active molecules like 4-Cl-IAA.

2.3.3. Binding Site Models

Kaethner anticipated the first binding site model.[132] His conformational change theory (CCT) was far away from a rigid hypothesis like the SCT (above). Kaethner proposed a "floor" of the receptor site, the responsible region for hydrogen bonding with the pyrrole nitrogen of IAA, which

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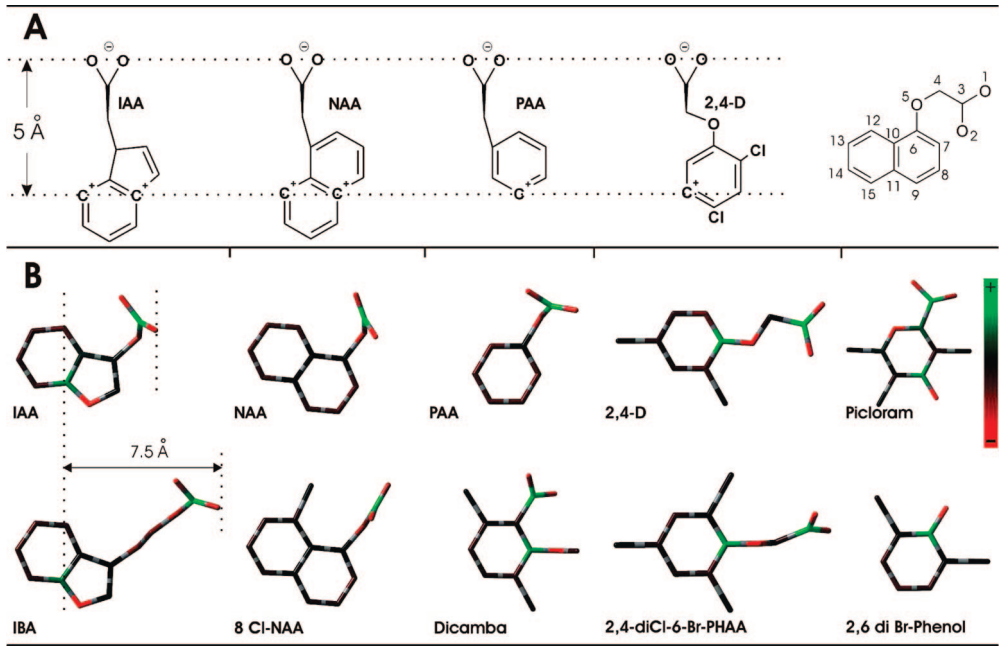

Figure 5. Thimann's SCT theory:[31] (A) separation of 5 Å between positively and negatively charged atoms as requisite for the auxin activity. (B) Calculated atomic charges based on Mulliken analysis are not in agreement. Green: positively charged atoms. Red: negatively charged atoms. 2,6-Br-Phenol is not a result of correlation between activity and the separated charges.[79] IBA is one of the most active auxin molecules with 7.5 Å of distance between positively and negatively charge atoms. Dicamba, 2,4-D, Picloram are potent auxins. For most molecules, it is not possible to find the most positive atom in the ring at longer distances with respect to the side chain.

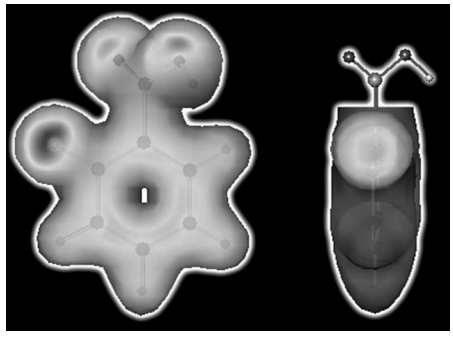

Figure 6. Surface activity ring theory supposes that the nonpolar part (basic ring system) plays the most important role to induce auxin biological effects. The representation of the electrostatic potential surface illustrates schematically one of the possible meanings of the surface activity proposed at that time. The electron cloud in the biological system is more important than the reaction of a specific atom or atoms. A functional group (carboxyl group) plays a secondary role and will be situated peripherically.[127,128]

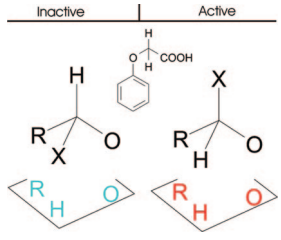

Figure 7. Three-point attachment theory illustrates that an active aryloxy acid has three functional groups (the unsaturated group, the α-H atom, and the COOH).[130,141] X and R represent the ring system and the carboxyl groups, respectively.

is consistent with other results.[31,46,79] However, Kaethner's theory was never challenged by experiments.

The model of Katekar,[51] frequently considered as the first binding site model,[131] was a result of an intuitive analysis supported by a systematic examination of approximately 20% of data accumulated by other authors. Katekar's model provided a more biological understanding than others. However, it is in conflict with Jönsson's realistic view: "it is so far too early to predict how these findings will influence the structure−activity discussions".[30]

The definition of Katekar's auxin receptor site is ex hypothesis complementary to the IAA molecule. His concept of an IAA receptor is inconsistent with the flexible scheme of molecular diversity in auxins and not supported experimentally anymore.[46] Katekar made this inconsistent concept of inflexibility even stronger during his further analysis based only on IAA derivatives for validating his deduction.[133–135] Kaethner and Katekar, however, introduced the pharmacophore concept in auxin-related research. Later, the growing capacity of computational chemistry permitted the use of new quantum-chemical methods in the study of auxin molecules. However, the last pharmacophore model[136] was focused on construction of a global binding site for auxins via the classical concept of hormone action. All three pharmacophore models are summarized in Figure 8. F8

Measurements of hydrophobic interaction abilities[121] as well as log P and log D indices[137] that fit the activity of a sample of molecules were announced as a new method to predict biological activity. Contrary to this result, Muir did not find any influence of the electronic and lipophilic effects of substituents on the ring related with a promotion of activity.[119] The lipophilic character of certain substituents was not substantiated as being a determinant factor for auxin activity. Auxin activity does not increase with increasing lipophilic character of the molecules. Molecules with similar lipid solubility have very different auxin activities.[31,125,127] The existence of auxin carriers[105,106] in the plasma membrane is another complex factor perturbing any kind of molecule-related activity analysis.

Further elaborations of the active molecule of auxin took into account deliberate design based on the existing biochemical knowledge. Binding assay results with ABP1 were

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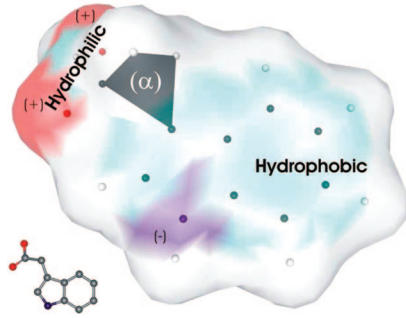

Figure 8. Schematic representation of the landscape of the pharmacophore model of auxin molecules based on the classic hormone concept. The positive and negative regions as well as the hydrophobic and hydrophilic region are shown.[132,136] The α carbon or buffer area is represented as well.[51]

used to establish requirements for auxins at the molecular level[81] and created new structure−activity approaches supposing ABP1 as the auxin receptor.[137,138] However, binding affinities between the auxin molecules and one ABPx are only one factor among several reactions to induce a biological effect. The existence of other putative auxin receptors is already confirmed and widely accepted.[46,58,63,64,106,139] The fundamental approach of individuality in the search for new drugs must be fulfilled.[140]

The proposed models until now are not generally suitable to explain experimental facts. This will be shown in a short summary: the TwoPA theory does not take into account the reversibility of enzymatic processes, the ThreePA theory does not consider benzoic acid derivatives, and SCT is not able to explain a multidimensional physiological phenomenon like auxin. In the case of CCT, first, the IAA binding site model does not deflexed the auxin molecular context and, second, a pharmacophoric model does not consider multireceptors. A more consequent explanation involves the surface activity at the electronic level depending on the balance of the substituents and the ring type. In spite of PCIB overemphasizing the influence of the specific spatial position of the COOH as a key feature, the almost forgotten theory (PCIB) is the one most able to explain the experimental facts in the last 20 years. The presence of molecular promiscuity in the molecular interaction with different proteins can only be explained by similarities in the surface of the electronic cloud of the molecules. This explains the activity of every auxin molecule without exceptions. However, a biological property based on the electron distribution of a small molecule is not a trivial problem to solve.

3. Consensus between Chemical and Biological Properties

The concepts of the dynamic regularity of the hormone–receptor interaction as well as the idea "one receptor−one ligand" have been imported from the animal model to infer results from plant bioassays.[141,142] In animals, a cell that has a predetermined competence to respond in a defined way to a specific hormone signal is called a target cell.[143] This provokes expression of a receptor gene with an inherent function for hormone action in the range of a few micromolar. In plants, however, every cell is a target cell for one or more of the plant hormones or other regulatory signals.[143] This is a direct reason for insufficient accuracy of the pharmacological methods applied to study plant growth regulation. The linear free-energy relationship (LFER) methods taking into account functional group contributions and the 3D methods, with positive results in pharmacology research,[144,145] have been elucidating the complexity of the auxin phenomena (see above). The plant growth bioassays are based upon the responses of the preformed organs, and the immediate stimulus merely "unblocks" some previous limitations. In action, the molecules in question operate as a part of a matrix of interacting and interlocking events.[141]

The auxin molecular diversity was not conceived among other biological facts inherent to plant biology, but the documented biological rules from animal hormones were used to innovate chemical dependences in fresh biological systems (plant system). The most likely solution of the problem is to consider, first, there is not a direct relation between chemical structure and physiological activity[127] and, second, plant molecular biology has changed the conventional reflections by the discovery of new molecular mechanisms in plants and even in biology.[46,64,146]

3.1. Plant Bioassays: A Poor Structural Mirror

The assumption "structure generates properties" introduced deterministically has created several speculative concepts about the molecular requirements of auxins. Went wrote in 1935 'of the different growth stages (initiation, differentiation, elongation, and maturation) elongation is the most spectacular and the one that can best be measured since it involves the greatest change in dimensions...'.[10] Went did not provide other evidence to select elongation as the main physiological event to focus on. Later, he concluded that the growth reaction is a chemical one. The meaning of 'chemical' was, however, not further specified.[127] Nevertheless, it was the base of many different types of biological tests, called 'auxin tests'.

One common description for an auxin effector, consistent with the nature of the auxin functions, is well accepted: an electronic-rich surface formed by different ring systems frequently combined with halogen substituents.[30,51,115,117,124,127,147] However, the historical background of plant auxin bioassays hampers examination due to irregularities in the results mainly because of (a) impurity of the used chemical substances, (b) lack of homogeneity in the output variables and their analysis, as well as (c) use of several nonstandardized different tests.[15,120,127,141]

In view of the numerous points which have to be considered in auxin investigation, the following strategies are recommendable for their biological activity analysis: (i) a consensus variable independent of test and tissue taking as a reference for the maximal activity of each substance[115] should be implemented. In addition, (ii) several substances tested in parallel by different assays combined with statistical multiscaling analysis.[79] Such approaches eliminate the redundant information focusing on the proper reaction. Thereby, they discriminate two groups of variables connected functionally with morphogenesis and growth (cell elongation)[79,86] (two different plant biological mechanisms influenced by auxins) as well as demonstrate the active function of noncarboxylic auxin substances.[79,148]

In fact, two sets of structural requirements make sense of the idea of 'a separate key to the back door' suggested for explaining the auxin phenomena[127] instead of the 'key−lock theory'. The general results suggest that the highest occupied molecular orbital (HOMO) localization on the *N*-indole atom plays a key role in auxin affinities for events like root induction. Morphogenesis (root induction) seems to be

dominated by ring interactions and by the recognized *N*-indole region.[79,126] The significance is supported by the observation that indole compounds act strongly on root induction but hardly on callus induction.[79] It can be postulated that callus formation depends on the mimetic representation of the orbital structure of the *N*-indole ring in other kinds of rings, which generates nonspecific interactions or is due to other interactions determined by the characteristics of the binding site.[46,79] A second region depends on the localization of the HOMO and/or HOMO-1 between the atomic positions ortho and meta with respect to the side chain (morphogenesis) and the atom in the position adjacent to the side chain bound to the carbon atom at the ortho position (growth).[79]

3.2. Auxin: Its Molecular Diversity and Pleiotropic Activity

A hormone messenger acts as an endocrine signal (Greek: "to secrete into"). This type of messenger is produced in a source tissue and carried by the circulatory system to its target tissues.[17] In plants there are independent units (no target tissue) without highly centralized centers to coordinate growth and development.[95] Therefore, several cells remain totipotent or can regain totipotency, and there is no clear division between germline and somatic cells, which infers differences in cell cycle regulation.[149] Diverse effects like cell elongation, growth, and differentiation are related to auxins. However, these processes do not share the same physiological pathways in the plant development strategy. Three main problems are connected with auxins.

(1) A high amount/number of active molecules. Affinity is described by the equilibrium constant for complex (AB) formation ($K_{eq} = [AB]/[A_{free}][B_{free}]$), the free energy of the complex formation is $\Delta G_{AB} = -RT \ln K_{eq}$. Specificity is conveniently defined as the difference in affinity between ligands A and A′ ($\Delta\Delta G_{AA'} = \Delta G_{AB} - \Delta G_{A'B}$). The original hormone definition does not include this specificity but specificity in effects;[150] this, conversely, is one essential characteristic for hormone−receptor interaction. A very high specificity requires stringent discrimination mechanisms when competitors are similar and abundant but flexible when competitors are few and distinct.[151] Auxins are dissimilar and abundant, a paradigm of hormone specificity,[152] or cannot be regarded as hormones.

(2) Pleiotropic physiological effect. Another controversial issue is the variable behavior of compounds strongly influenced by the type of assay performed with different plant species and organs, e.g., IAA is 1000 times more effective in the Avena curvature test than 2,4-D, in the split pea test 2,4-D appears to be 12 times as effective as IAA, whereas in the straight growth test IAA and 2,4-D have comparable activities. It is very difficult to follow the proper reaction from a phenomenological point of view by a single biological test. The auxin specificity depends on its induced effect. Consequently, it is not known if the causal properties of the molecules have been adequately addressed in this context or the characterization of the respective effects in different tests.[141]

(3) The high molecular diversity is a very crucial point, since most structure−activity theories on auxins have not been proven by well-defined and reproducible experiments. Strategies for the selection of chemical descriptors that handle such different structures and association of the molecular individualities are a difficult task. There are several pieces of experimental evidence with insufficient statistical representation, and the experimental samples are affected by the influence of different experimental errors associated with different authors and experimental methods as well as intuition as the base of the reflections.[31,51,117,125,132,136] Reliable experimental data are highly needed, but at present it is not feasible to generate them for such a high number of compounds.

Other issues related to pleiotropy complicate SAR models of auxin. Auxins are transported intercellularly and perhaps intracellularly (via whole families of proteins that are parts of transporter complexes that are not well defined), undergo a broad range of conjugation events (to sugars and amino acids to name a few), and are turned over within cells or related with other metabolic pathways like glucosinolates.[153] Thus, the presumed differential effects of auxins in some assays may not be related directly to perception and response. Rather they could be related to local availability of active auxins.

4. Chemical Similarity: The Necessity of a Statistical Approach

The current section is endeavoring to state as briefly as possible the issues which seem to be involved with a more complicated task for the already complicated two general classes of QSAR models oriented to (1) nonspecific inhibitors whose inhibitory action can be correlated by Log P alone or (2) inhibitor active site of one enzyme.[154] The pharmacophore concept (the mapping of common structural features of active analogues that bind to the same receptor[155]) cannot overcome the structure−activity impasse of the auxin signals in terms of one receptor. The idea of the drug−receptor interaction like a key fitting a lock is too simplistic and limited to few applications.[156] The molecule (key) is a flexible entity, and their study in nonactive environments may lead to the incorrect conclusions. Therefore, the expectation that the detailed study of the small molecules with one foreseeable crystal structure of a protein is not always appropriated.[157] In auxins, there are further flexibilities of the lock entity affecting the basic philosophy for receptor mapping.

Structural distinctiveness or resemblance among auxins must be elucidated by statistical regularities as expression of a more basic dynamic regularity[158] of supramolecular soft bonds influencing biological behavior. As a physiological consequence (a) the term "sensitivity", which implies availability of a receptor, is a new factor in plant hormone signaling.[87,88,159] Next, in terms of ligand−protein interaction (b) the promiscuity of auxin−protein interactions encompasses repercussion on the electronic rather than atomic rearrangement.[46,160]

The ligand−protein assemblies of auxin-like molecules in both TIR1 and ABP1 binding sites are completely different.[46,160] First, the ABP1 binding site is localized at ∼11 Å from the protein surface, while the auxin molecule on TIR1 serves as 'glue' for sticking two protein surfaces in the complex Skp1-Cullin-F-box-protein^{TIR1}-IAA7. Second, the pharmacophore concept cannot be taken into account even in terms of consensus protein binding site because few hydrophobic amino acids such as proline, leucine, and phenylalanine are common in both binding sites. ABP1 has three histidines, phenylalanine, glutamine isoleusine, and tryptophan as well

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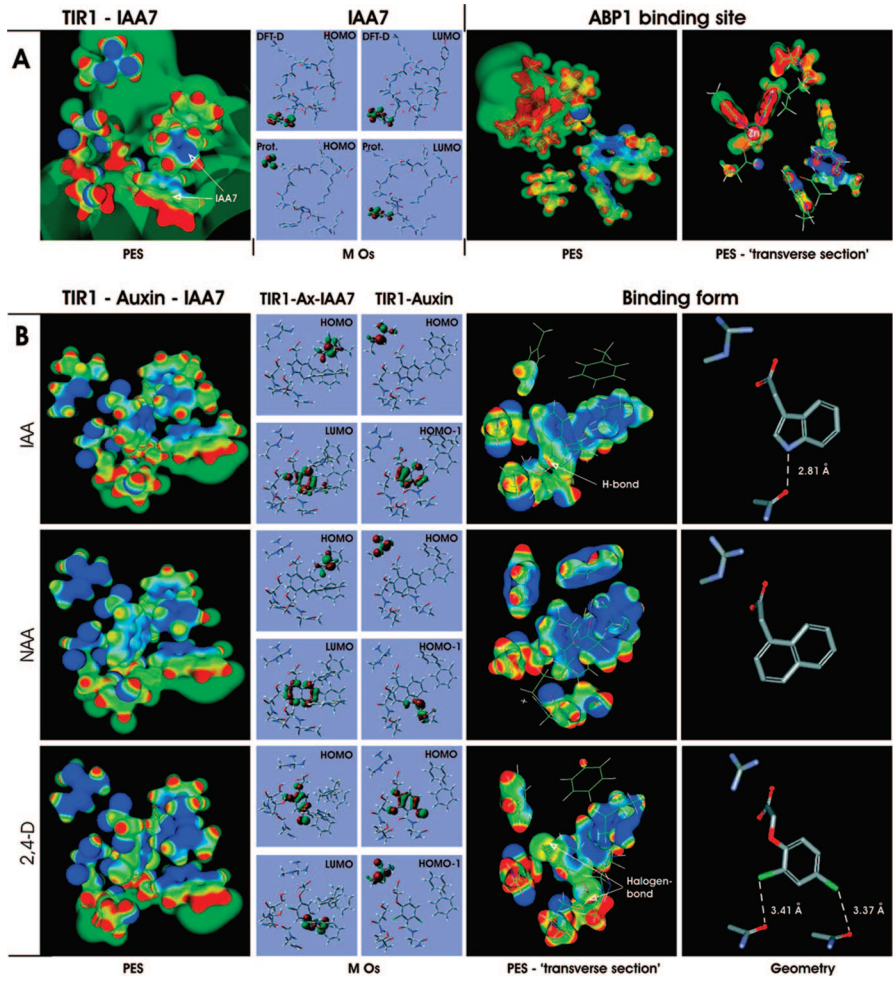

Figure 9. Molecular surface electrostatic potentials on the auxin binding sites. (A) Difference between the electrostatic potential of both the ABP1 and the SCF^{TIR1} binding sites without the auxin molecule computed at the B3LYP 6-31G* level. The protein IAA7 was used as in the native geometry in the binding site and was also optimized at the BLYP-D 6-31G* level. The HOMO and LUMO orbitals were computed at the Hartree−Fock 3-21G level. (B) Differences on the electrostatic potentials and outer molecular orbitals (HOMO and LUMO) due to binding of different auxins (IAA, 1-NAA, and 2,4-D) in the SCF^{TIR1} binding site computed at the B3LYP 6-31G* level. Color range in kcal/mol: red, more positive regions; blue, more negative regions.

as a transition metal (Zn) that forms a complex with the carboxyl group of the auxin's side chain. The SCF^{TIR1} binding site is formed by valine, serine, glycine, and arginine, while tryptophan belongs to the IAA7 peptide. The standard deviation of the VdW volume in the TIR1 binding site is 10 times higher than in ABP1. It suggests that the binding site of one small auxin-like molecule is determined by some specific amino acid distributions able to control the thermodynamic (minimize the ΔG) rather than the kinetic equilibria.

4.1. Binding Site at the Electronic Level

Analysis of the electronic architecture (Figure 9) by quantum chemical analysis at the DFT level with B3LYP/6-31G of the crystallized protein complexes SCF^{TIR1}−auxin and ABP1−auxin[46,160] shows the electronic basis of the auxin binding. The surface electrostatic potentials of both binding sites shows differences (Figure 9A). The SCF^{TIR1} binding site has an almost uniform potential pattern without the auxin molecule. This suggests that the auxin molecule generates potential differences in the SCF^{TIR1} binding site which already exist in the ABP1 binding site.

The binding mechanism of ABP1 is still not clear at the molecular level, and the proposed coordination structure needs further chemical evidence.[161] A theoretical model for ABP1 suggested a conformational change of the protein to fit the auxin ligand into the binding site involving a metal ion.[162] This was partially confirmed by experiment. The binding to a metal ion (Zn) was confirmed experimentally, but the proposed conformational change has not been found in any crystal of auxin binding proteins.[46,160,163] This proves also that a small molecule, which is not able to form several H bonds, is unlikely to alter the atomic structure of a large molecule as a protein. The stability of the complex with the Zn atom and its physiological impact must be analyzed as well as the structural influence of surrounding water molecules.[164] The physiological conditions of the medium for binding under extracellular conditions (pH = 5.5) do not favor the existence of auxins with a negatively charged carboxyl group. However, the physiological involvement of ABP1 is, in principle, unquestionable, and normally such coordination is related to redox reactions.[165]

The complex TIR1-Auxin-IAA7 is evidence of an auxin-mediated hydrophobic interaction between nonhydrophobic protein−protein interfaces. The usual composition of polar residues of histidine, tyrosine, and phenylalanine in the protein interfaces is not present.[166] The stabilization of protein−protein association upon the binding of auxin-like

molecules leads to a more hydrophobic interface due to the gain in free energy.

Identification of molecules able to fit in the binding site is related to information regarding the nature of the intermolecular forces involved in determining the biological or other activity of the compounds in question, which is studied with several intermolecular descriptors.[167] The existence of more than one putative receptor for IAA, which additionally shares the same binding site with molecules such as 2,4-D, was unclear until the crystal structure of SCF^{TIR1} was revealed.[46] TIR1 provides the flexibility of molecular conjugations with IAA, 1-NAA, and 2,4-D. The transverse section of the PES (Figure 9B) shows hydrogen bonding due to the *N*-indole (IAA) as well as the halogen binding of 2,4-D, while 1-NAA does not show any kind of such interactions. In general, π-stacking interactions play a decisive role in this binding site. Thus, the bulk polarization energy in π-conjugations is associated with the electron density deformation that occurs in the molecular boundaries due to electron transfer.[168,169]

As already known, only a significant number of H bonds is determinant to induce interactions (e.g., five H bindings). Therefore, in auxin molecules a simple COOH group will be under the domain of a ring influence. Electrostatic forces, exchange repulsion, and mutual deformation of the electron densities of interactions of the molecules in question[169] make it possible that 1-NAA minimizes the *N*-indole interaction[170] of IAA to form a H bond while 2,4-D forms two halogen bonds.

The idea of "molecular promiscuity" in TIR1 was predicted due to similarities in the electron densities[115] as well as their implications on the biological activity.[79] These clarify experimentally (a) unspecific interactions between auxin and proteins, approached by Ferro et al. previously,[79] and suggest (b) the existence of more than one kind of binding system in the same binding site. The so-called "molecular promiscuity", in terms of 'unspecific' or statistical molecular interactions, is an expression of fragile bond formation between the auxin molecule and its receptor, which has common prerequisites of interactions in the cellular medium for different auxin-like molecules. These fragile bonds can be classified as a delocalized dispersion interaction (weak interactions of the VdW type) which is not well described with the present quantum chemical methods. Their weak energy changes are overlapped by other interactions like hydrogen bonds. Currently, some new theoretical methods are developed for solving such problems.[171]

The low binding constant of the auxin−protein interaction[58] suggests considering also the small values of K_A ranging from 10^{-3} to 10^{-6} M for the electron transfer reaction.[172] Formation of the so-called "charge transfer complex" for auxins[51,173] is also highly dependent on the ion strength[60] and does not assume to be dependent on the size of the interface areas.[172] "Charge transfer" is defined as the movement of electrons from molecule X into the unoccupied molecular orbitals (MOs) that are part of the molecule Y subsystem and vice versa.[169] Essentially a mechanism of charge transfer is always fashioned by a system donor–bridge–acceptor.[174]

The TIR1 protein acts as one side of a donor−bridge complex in the binding site for the auxin molecule. The free side chain of the auxins interacts with the positive fraction of an arginine (forming a 'salt bridge'). This fraction is related to the localization of the HOMO orbital in the binding site (Figure 9). Arginine is one of the most polar amino acid residues which produce stable hydrophobic interactions with the heteroring of the tryptophan mediated through the methylene groups.[107] Its long side chain is frequently found in vigorous motion, a reason for the binding of different auxin molecules, and there will be an entropic penalty when this flexible side chain is so firmly pinned down. The second region in the auxin binding site of TIR1 is formed by the main chain (backbone) of some amino acids like leucine and two serines. These are classical polar protein regions able to participate in hydrogen bonding. The last region is formed by electroneutral side chains.

Figure 9B provides evidence of different intermolecular interactions of auxin molecule species in the same binding site. The calculated electrostatic potential of the binding site surface changes similarly after the binding of the three auxins (see the difference: Figure 8A without auxin and Figure 8B with the auxins). The obtained work per unit charge favors the spontaneous move of the negative charges toward the positive region localized around the tryptophan, which belongs to the IAA7 protein (the other side of a donor−bridge complex) (Figure 9B).

Previous analysis of the energy of the highest occupied molecular orbitals came to the conclusion that HOMO and HOMO-1 form an active energetic quasi-band,[79] which is able to explain the biological activity of auxins as it was revealed in other biological systems.[175] On this basis, the representation of the MOs in the binding site for the complexes of TIR1−auxins was performed (Figure 9). For the complexes TIR1−1-NAA and TIR1−IAA the HOMO corresponds to the fraction of the arginine and the HOMO-1 corresponds to the auxin molecules. For the complex TIR1−2,4-D the HOMO is localized on the 2,4-D ring system and the HOMO-1 on the mentioned fraction of arginine (Figure 9B). The differences in the HOMOs localizations at the binding site are influenced by specific attractive forces among the atoms involved in the binding site. Halogen atoms (like Cl in 2,4-D) form intermolecular interactions with atoms containing lone pairs, where the electron transfer occurs from the donor site to the halogen atom.[176] This facilitates the electron transfer toward 2,4-D.

The protein IAA7 shows its lowest unoccupied molecular orbital (LUMO) localized on the tryptophan that shares the binding site with TIR1. Optimization of IAA7 at the density functional theory (DFT) level with dispersion parametrization (DFT-D[171]) confirms the presence of the LUMO in the same region (Figure 9A2). This tryptophan describes the easiest route to the addition of electrons to the system for the IAA7 structure at the binding site and at the optimized structure with DFT-D. It suggests that IAA7 is the electron acceptor in the system.

The molecular functions of the auxins are far too many to be concluded in a few pages, and the two protein binding sites discovered until now only open the scope for additional much more dynamical ways of interaction in plants. Different methods can be used like ab initio ligand design to determine the free energy of the binding by calculation of the H bond, ionic, lipophilic, and rotatable energies. In addition, calculation of the components of the thermodynamic cycle for the complex formations like electrostatic interactions, the cavitation free energy (dispersion and electrostatic energies), loss of side chain conformation entropy on binding, VdW interaction, and loss of translational and rotational entropy can be carried out.[177] Single analysis of each crystal structure

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will only result in information on particular interaction properties. These factors (properties) are influencing the general effects of auxins. It can be expected that the effects of these factors are highly correlated. This complicates any further theoretical analysis. At this point it is important not to forget the biological roots and design rational experiments considering different auxin−proteins complexes and its biological implications.

4.2. Signal Hypermolecule in the Active Environment

The use of the organochemical intuition and pharmacophore approaches, in principle, has been withheld a priori judgements about the structural causes being responsible for the biological functions. The structure−activity relation in auxins was approached by Hansch,[178,179] the creator of the Hansch method in quantitative structure−activity relationships (QSAR). His approach[180–182] has been successfully applied to predict substituent effects in a wide number of biological assays but essentially failed to produce coherent results for auxins. Later, biological activities of phenol derivatives were misinterpreted by the mimetic effects between the nitro and the carboxyl group[116,124] because the significantly higher difference in the electron correlation effects at the quantum level between both COOH and NO_2 was not considered. Other phenol derivatives, like 2,6-Br-phenol, have been found to be much more active than nitro derivatives (inactive) in different tests.[79]

Molecular space defined at the geometric 3D-space level of a receptor[132,136,183] does not explain the auxin phenomena but partial interactions. An analysis in the case of auxins cannot be functionally focused on a "one receptor−one molecule" interaction, not even on "one receptor−one type of molecule". The hypermolecule is defined here as the group of molecules without statistical differences among their molecular wave functions (Figure 10).[79,115,184] Different kinds of interactions in the cellular or extracellular environment need specific requirements from a "matching sample" of molecules (hypermolecule) depending on the molecular acceptor and its environment. Each binding system includes the receptor and all different molecules able to bind to the receptor generating the hypermolecule or "master molecule" (pool of binding molecules). The binding of different auxins with different receptors increases the probability of reinstatement based on the probabilistic molecular membership of the hypermolecules, which generate dynamic interactions guided by the intermolecular patterns of each auxin−receptor system.

Analyses of the quantum matrix of similarity index show repetitive information,[115] which depends on the set of molecules used (statistical sample). It prevents an extrapolation of results coming from a small sample of molecules to the general auxin phenomena. Auxins play many important roles in biological systems as essential functional and/or structural cofactors in proteins. They can save energy needed for conformational changes in the formation of protein complexes or other requirements related to electron arrangement.[46] To prevent incorrect conclusions the analysis of auxin activity must be focused on facts placed in the active environment. A posteriori statistical techniques based on the phenomenological context are needed to focus on the interface between chemistry and biology.

Nevertheless, linear dependences between structure and biological activity are forgoing the biological roots. Biological networks exhibit emergent properties such as integration of signals across multiple time scales and generation of distinct outputs depending on input strength and duration.[185] On the basis of the natural principles chemical reactions and cooperative interactions are nonlinear. The cooperativity always implies nonlinearity in the response of the system to the first signal. Therefore, we can concentrate on some minimal requirements of the molecular structure that are able to switch the onset of biological processes.

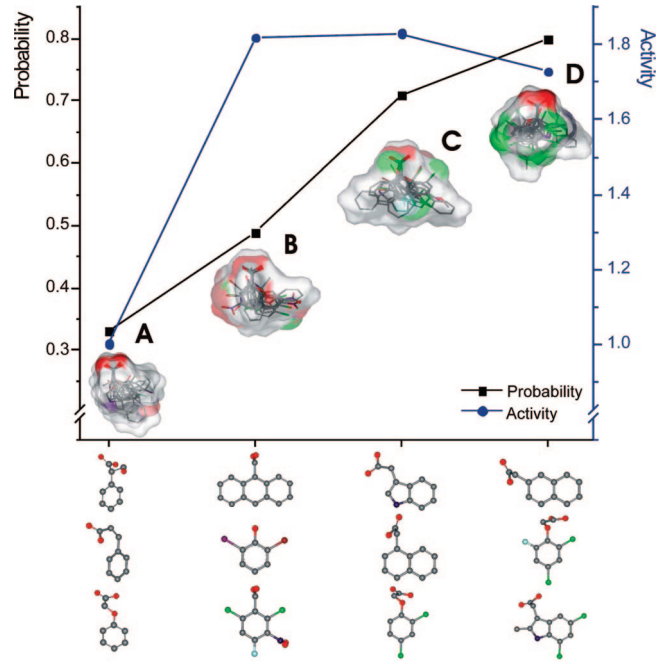

Figure 10. Probability to find active molecules in each group of similarity is shown as well as the average of the activity of the active molecules obtained by information from the literature.[115] In the graphic are represented the four different auxin hypermolecules with biological impact generated by quantum molecular similarities. Underneath are three molecules representative from each group. The average for each group of molecules was determined as follows: $\sum$(consensus activity)/N(molecules per group). The activity is defined as follows: 0, inactive; 1, low activity; 2, active; 3, very active.

According to Gafurov,[186] weak but simultaneously stimulating influences on some systems with a given character can cause a strong response in case that the physical and chemical properties of the effector fragment and the whole molecule themselves do not determine the regulation influence qualitatively. We are facing a phenomenon where the cause−effect relationship is a statistical regularity. This means that the efficient cause of the effect depends on several functions (the incidental cause is indeterminable) to realize the same state of the system, which generates the same sequence of states as responses.[158]

Because of the level of randomness of the auxin targets, the evaluation and manipulation of the chemical space defined by several hundreds of auxins is a theoretical impediment[187] for a definition of a global response surface. In a recent theoretical study it was found that quantum molecular similarity measures (QMSM) are correlated with molecular volume and the charge distribution on the molecular surface have satisfied some biological requirements of activity at the experimental level.[79,115,175,184] Their applications have selected common features of the individual molecular wave function. Principal component analysis (PCA)[188] eliminates the repetitive information of the quantum

variables and eliminates the phenomenological superposition of the biological effects. The PCA contains the distinct features of the molecules analyzed at the population level. These features represent the dissimilar abilities of intermolecular interactions provided by a description of the hypermolecules.[115] Consequently, the ruggedness predicted in the molecular similarity would then be related with the different binding landscapes of the related network and therefore necessary for different biological activities and/or their levels (Figure 10).

The results show four important groups of molecules differentiated by their wave function (Figure 10). The probability to find active molecules at different levels of similarity is shown in the graph as well as the most representative molecules per group (Figure 10). IAA, 1-NAA, and 2,4-D belong to the same group (C). If we consider experimental data from the literature[30,51,115,116] (Figure 10), the quantum chemical similarities defined the most probable electronic frame of an active molecule but do not quantify its activity.

Qualitatively the analysis of auxin-like molecules provides an objective direction by QMSM. As predicted, induction of auxin-like activity proposed for some compounds in the literature was not confirmed by fresh data for group B.[79] The inactivity of molecules like 8-Cl-NAA, which cannot be explained by classical structural requirements analysis,[135] could be identified by quantum similarities. Alternately, the activity of phenol and benzoic acid derivatives was confirmed and explained.[79,115] In addition, the separation between the first two groups (groups A and B) with a tendency to be inactive and the last two groups (groups C and D) with a tendency to be active has found experimental bases[79] (Figure 10).

The distance between the carboxyl group and different ring atoms as a wider viewpoint of SCT had no statistical influence on the activity for the sample analyzed.[79] However, the balance between the hydrophilic (carboxyl group) and lipophilic regions (ring system)[189,190] was found to be important. The so-called buffer area (sp^2 carbon between the ring and the carboxyl group) postulated by Katekar[133] is a determinant variable for the occurrence of high specific activities, especially cell growth.[79,191] Besides the QMSM, as an intermolecular descriptor, the electronic descriptors like frontier MOs are a fine complement.[115,175]

Quantum chemical calculations represent the only way to describe molecular properties a priori and to explain the physical and chemical state of the hypermolecular system.[192] Understanding a molecule only as a skeleton made of atoms is obsolete; instead, molecules must be considered as a distribution of an electromagnetic field between electrons and atoms. When the small molecule approaches the active site of the receptor molecule, it interacts with an electron distribution, not with a set of hard-sphere atoms.[157] The large amount of different auxin-like molecules and the existence of multireceptors in the auxin phenomena lead to more than one hypermolecule.

5. Receptor: Evolutive Implication and Intramolecular Protein Interactions

The tiny fraction of the biologically relevant organic chemical space[193] imposed a reduced molecular world with increasing particularities and therefore interdependences between variables coming from specific atom associations and the biological environment. Therefore, the influence of certain chemical bonds cannot induce a determinant effect in the background of networks of biological signaling pathways.[45] Rather, they can influence probable paths within the randomness of the weak intermolecular interactions.

The underlying idea could not be developed in the first half of the 20th century under the nascent concepts of hormone and receptor as well as the theory "one gene−one enzyme" from Neurospore in 1958. The second half of the 20th century set up new views about the chemical interactions in biology having as background the work on the dynamics of the genome of Barbara McClintock. As late as the 1990s the paradigms changed with the works "one gene−many proteins" and "signal transduction" (both Novel price in 1993 and 1994, respectively). This new level of research gives other opportunities to analyze the auxin phenomenon.

Substances that have the capacity to evoke some specific reactions became hormone or signal molecules if they did not already have another evolutionary purpose[194] and if they were able to bind to receptors to transmit information into a cell without an endocrine system. *Tetrahymena* recognizes amino acids and the hormone derived from the amino acid as similar molecules.[195] Therefore, a chemical−biological unit like IAA, as a derivative from tryptophan, set a double condition of interactions: (1) recognition of the indole rings is predetermined in proteins structure and (2) carboxyl group recognition in the cell.

According to a reappraise of the phytohormone concept, plant hormones may act through several distinct roles, or a combination, according to the physiological phenomenon considered.[196] This implies a necessity of an evolution of the receptor concept, increasing its flexibility view.

Analysis of the geometric packing (depending stacking interactions by means of π electrons) of the tryptophan residues in the protein follows geometric patterns of interactions with other amino acid residues.[197] These interactions are related to the spatial distribution of the frontier molecular orbitals and the quadrupole moment of the amino acid residues guided by a solid backbone of the protein. If the electronic structure of the indolic ring of both tryptophan and IAA is conserved,[79] the free IAA molecules, mimetizing indole stacking interactions, might find new sites (gaps) of coupling in the proteins. Under these conditions other carboxylated rings are also able to interact mimetically with such cavities in the proteins. Even other noncarboxylated rings showing a similar surface active area.

However, application of the classical view of mass action as a receptor−stimulus model, which was dominating until the beginning of the 1980s, only considered the property of the substance in isolation. Any suggestion about the structure function of auxin could have been demonstrated scientifically. Only the receptor−transduction models are able to take into account a substance's chemical information.[198] Thus, the efficacy is attributed to chemical binding and together with affinity can be associated to the chemical properties of the substances and thus be incorporated into structure−activity relations.[198,199] The new view about the binding of a ligand to the receptor is not a simple process of binding and occupation but a kinetic process in which molecules move toward and away from the receptor at various rates.[200]

In this context, mechanisms to understand the modulation of interactions with the carboxyl group in auxins can be explained. The oxygen present in the carboxyl group provides a molecular tool for recognition. Singlet oxygen might quench the reorientation of the chemical groups due to

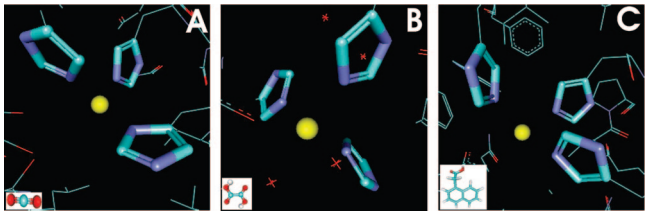

Figure 11. Schematic representation of the binding sites for the protein carbonic anhydrases (A) [PDB 2FNM], Cupin 2 Domain (B) [PDB 3IBM], and ABP1 (C) [PDB 1LRH]. It shows the similar structure of the complex of three histidines with the Zn (yellow sphere), the responsible ion for coordination of the carboxyl group in auxin. The three proteins are related to CO_2, oxalic acid, and NAA (these structures are represented as a small figure in the left corner of each schema), respectively.

stereoselectivity trends on one side of a molecule but react chemically on the other. Such property might help molecules target specific sites in the substrate.[201,202]

One example of such a conserved kind of interaction with oxygen is the central structure for function management composed by three histidine residues coordinated with a Zn ion.[203,204] Such active sites are naturally occurring zinc prosthetic groups in most of the carbonic anhydrases in animals and plants. The function of this family of metalloenzyme is the catalysis of the rapid conversion of CO_2 to bicarbonate and protons. Another example is the family of proteins (cupins) that includes germin and the vicilins.[160] These proteins are strongly involved in oxalate metabolism.[205] ABP1 with two sequence motifs, HXH(X)11G and P(X)4H(X)3N, belongs to the cupins proteins and has a similar structure for recognizing the carboxyl group of auxin[160] (Figure 11). This suggests that ABP1 inherited a structure evolutionarily existent for identification of oxygen atoms (as CO_2 or R−COOH) to recognize auxin-like molecules.

The abundance of IAA in many different biological organisms, even as a signaling molecule in bacteria,[37] suggests a coevolution of pre-existent interaction recognition systems. However, IAA has acquired signaling properties for growth and development only in plants.[123] It can be speculated that ABP1 is an expression of the most direct evolution of the COOH recognition system and TIR1 originated from the evolution of the identification of the indole pockets. This hypothesis, however, does not exclude other kinds of interactions.

6. Conclusions

The view of ligand-like auxin changed from a generic concept as plant correlation carriers[4] to a chemical definition of a set of chemical structures under the inappropriate hypothesis of one macromolecular target (receptor) in the cell. However, a multireceptor mechanism of action involving different signal systems is already proven by phenomenological,[79,127] biochemical,[15,59,61] and structural[46,160,184] data.

The flexible nature of molecular interactions in biological systems is definitively a recent field of analysis and not a peculiarity of auxins: (a) Cytochrome P450 has ligand promiscuity due to the existence of multiple binding modes,[206,207] and (b) experiments on the gene silencing activity of siRNAs with a ribo-difluorotoluyl nucleotide revealed that the stacking interactions play a major role in the fidelity of DNA replication rather than hydrogen bonding.[208,209] Therefore, it can be summed up that the variation of the chemical interactions is a consequence of the trend of the evolving biological system under supramolecular equilibrium.[210]

The biological viewpoint of finding receptors is unclear in the auxin phenomenon. The term "auxin binding protein" has been defined without a scientific account of the "auxin term". ABP1 is not able to generate any correlation with the gene expression and has not been involved in every auxin effect until now (see, for example, the reviews in refs 68, 139, 160, and 211). TIR1 was originally identified because its mutations result in resistance to inhibitors of polar auxin transport, and these mutations were subsequently found also to confer auxin resistance. However, the facts do not necessarily prove that TIR1 is involved in all auxin functions.[68] Other recent publications refer to auxins as a glue molecule to activate a protein complex required for cell division as well.[212] Both systems (ABP1 and SCF^{TIR1}) are not able to explain the most significant activities of auxins, the short time responses.[68,213]

6.1. Entropic Processes in the Auxin Machinery

Experimental data show that the variance of the response variables is inversely proportional to the increment of the hormone concentration (Figure 12). The decrement of the variance means that the inherent biological variation of the system (tissue) has changed (diminished). This change of the system can be described by the entropy, and the relation between variance and entropy is determined essentially by the central limit theorem[214] as proportionally inverse.[215] Physiologically, primary auxin effects, like a change of the consistence and permeability of the plasmalemma, also involve all kinds of secondary effects and those in particular might influence metabolism and growth. The gradients of entropy and dimensionality from disorder to symmetry suggest that entropy maximization creates complexity as partial symmetries (periodic and chaotic attractors, dissipative structures, organisms, etc.).[216]

The auxin molecule as a bit of information increases the thermodynamic entropy because it increases the number of possible microscopic states, thus making any complete state longer. Processes like morphogenesis require assembly of protein subunits to form noncovalent aggregates. Such aggregate formation is endothermic and energetically favored by an increase in entropy.[217] If the processes are irreversible, the thermodynamic manifold required must be appropriately increased in dimensions.[218] This supports the auxin activity decrease with the reduction of the variance trend patterns while the informational entropy (Shannon) increases until an optimal point[216] (Figure 12). However, some peculiarities in the activity depend on the type of auxin molecule (Figure 12). The most active and known auxin molecules (group I) induce action at a concentration of 10^{-7} as reported,[79,191,219] and they reveal a tendency to maximize the entropy in callus and root induction as well as minimize the variance in the root inhibition at a concentration of 10^{-5} M. The molecules in group II are represented by a group of molecules with a tendency to induce callus and less root induction. Group III contains almost inactive molecules.

A dose−effect relationship regarding callus induction is not observed for different active auxin molecules but only for those not able to inform the tissue satisfactorily. A degeneration of the propagated action of receptor occupation suggests a random growth (callus). At high concentrations multiple responses occurred that were able to uncoordinate

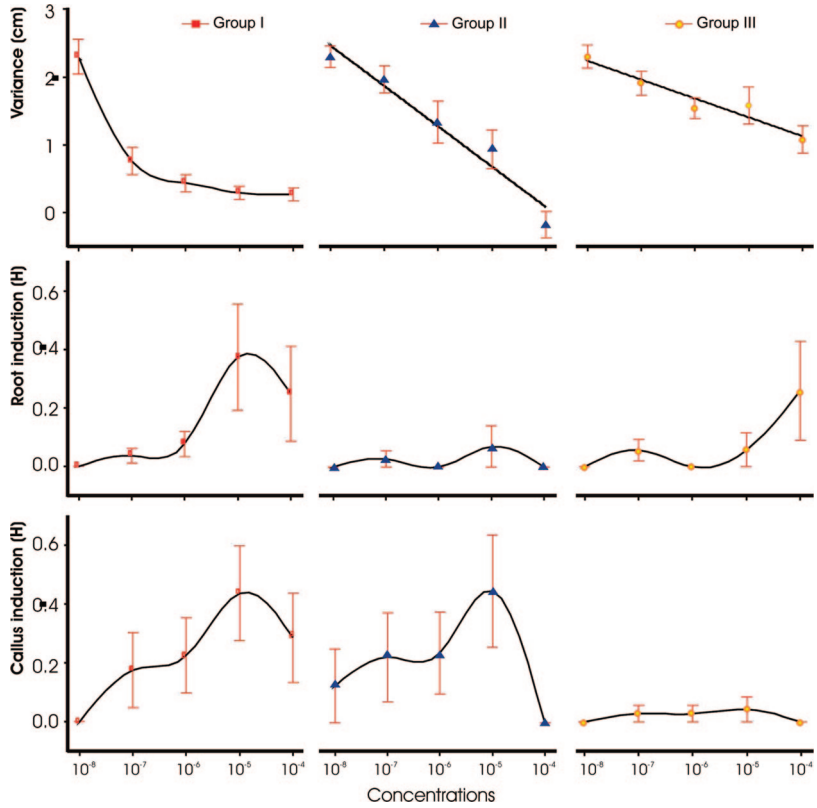

Figure 12. Behavior of the variance of the biological activity with respect to the concentration of different auxin-like molecules. The molecules were clustered by means of analysis of their variances using the Ward method with Euclidian distance. The three lines represent different groups of molecules with or without biological activity. Group I is representative for the most known auxin compounds [IAA, 2,4-D, 1-NAA, Dicamba, IBA, 2,4-Br-PHAA; 2-F-BA; 3-F-PAA, 2,4-diCl-PAA, and 2,6-diCl-PAA]. This group determines the response of the tissue starting from a very low concentration, and the curve of variance decreases slowly. Group II [Picloram; 2,6-Br-phenol; 2,4,5-T; 2-NAA, Skatol, 3-Me-PHAA; 2-nitro-PHAA; 1-naphthoicacetic acid and 2-naphthoicacetic acid] follows the linear response of the variance, and the major influence is visible in the highest concentrations. On the contrary, in group III most of the molecules are inactive in this test [TIBA; ILA; Trysben; PHAA; indole-3-acetamide; 2,6-nitro-phenol, and 2-Cl-6-nitro-phenol]. The comparison shows that all the active molecules are not able to produce the same pattern of control. Shannon entropy (H) for the morphogenesis process in vitro (callus and root induction) was calculated ($-1(-1\{[p(x_i) + 0.5]\,[\log (p(x_i) + 0.5)]\}$) and analyzed statistically.

the normal growth and development as found for 2,4-D.[97] The induction of phenomena like callus is explained by convolutions due to the inability to use the energy in the system due to unspecific interactions.[214]

The nonadditive factors involved in the auxin responses are related to the (i) specificity of the different substances, (ii) reactivity of the different substances, (iii) concentration as measure of potential energy, and (iv) response of the system (plant or tissue) as feedback.

In biochemical terms of the law of mass action, assuming that the ligand is in excess with respect to the receptor, the yield ratio of the equilibrium concentration depends on the number of bound and free ligands. A high affinity implies that most of the ligands are in the bound state and that the bound:free ratio is large. Then the dissociation constant (K_D) must be small relative to the receptor, which indicates that affinity depends on the ligand concentration as well as on K_D. Thus, affinity is a relative concept, not an intrinsic property of the molecule.[220] The dependence between the auxin molecular structure and the biological properties ("specificity") can be clarified by the use of two different kinds of entropies (thermodynamic and informational). At a practical level, it is necessary to explain which properties produce informational entropy (measure of the uncertainty associated with a random variable; defined in the context of a probabilistic model) and which produce thermodynamic entropy (a change to a more disordered state at the molecular level; a system spontaneously evolves away from its initial conditions).

It is also necessary to consider that the molecular reactivity expressed in the chemical hardness (η) plays an important role in auxin processes,[79] especially if we consider that soft reactions are common but hard−hard product are more stable.[221,222] It implies the existence of ligand competition to react in the biological system and give an explanation for the existence of different pathway activation.[223] Other differences or irregularities in the activities of group III could be explained by regulation of the tryptophan pathway.[224] Molecules (group III) like indole-lactic acid (ILA) and indole acetamide influence the root induction only at the highest concentration (Figure 12, group III).[79,225,226]

Quite apart from the complexity of the auxin signal system, the combination of both molecular electronic structure and intermolecular interaction descriptors leads to identification of molecules with a similar surface charge distribution, which generates promiscuous recognition. Despite the fact that 1-NAA is not able to form intermolecular bonds, like a H bond with the *N*-indole in IAA or halogen bond in 2,4-D, stacking interactions are playing an important role in the recognition of the auxin's ring system.[46,64]

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7. Perspectives

A strategy that incorporates information about the chemical structure on the design of experiments into the systematic accounts of the subject, the analysis of both, a complete set of auxins chemically and the phenomena functionally is key for generation of new synthetic products and comprehensive explanations for the action mechanisms. The crystal structures of ABP1 and TIR1 do not reveal the action mechanism of auxin-like molecules entirely, but they represent a well-defined starting point for analysis of their different recognition complexes using molecular modeling methods.

A feedback approach using computational chemistry combined with molecular biological methods represents a promising strategy to analyze pleiotropic effects of phytoregulators (auxins, gibberellins, brassinosteroids). The impact of molecular similarities of auxin molecules[79,115] has been already taken into account in a study on the mode of action of auxinic herbicides,[227] as well as the current issue of the implications of auxin (IAA) signaling in plant defenses due to the transport and reception of bacteria.[37,38,228] However, data analysis must be based on accurate statistical analysis to solve the indirect relationships between molecular structure (cause) and biological activities (effects). The new variables are strongly linked to fresh biological evidence, thus permitting expanding the classifications of auxin-like molecules. This will unravel the connections at the biochemical and molecular levels. Other molecular properties (e.g., reactivity with hardness) calculated by quantum chemical methods have been related to auxin activity for the first time recently.[79] The localization of the molecular orbitals on specific atoms on the molecule and the existence of the sp^2 carbon in the side chain must be analyzed carefully by accurate methods such as DFT for a broad number of molecules. Furthermore, a more detailed exploration of molecular quantum similarities is needed.

The existence of more than one receptor in the chain of auxin signal transduction demands the discrimination of the structural-binding relationships for each receptor−ligand pair and evaluation of the physiological relationships for each of them. Further nonadditive influences from a molecular and biochemical context can be expected.

Classification of the hypermolecules is the approach to define the auxin system. There is a high demand for further analyses, like fishing proteins in the cellular system responsible for the activities of different auxin conformers. Accessible methods for this task, like the combination of surface plasmon resonance (SPR)-based technology with mass spectroscopy (MS), have already been described in the literature.[229]

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