Chemical biology and digital image processing to unravel complex molecular mechanisms in *Arabidopsis*

Thomas Vain
Faculty of Forest Genetics
Department of Forest Genetics and Plant Physiology
Umeå

Doctoral Thesis
Swedish University of Agricultural Sciences
Umeå 2016

Acta Universitatis agriculturae Sueciae
2016:104

ISSN 1652-6880
ISBN (print version) 978-91-576-8711-1
ISBN (electronic version) 978-91-576-8712-8

Print: Arkitekopia, Umeå 2016

Chemical biology and digital image processing to unravel complex molecular mechanisms in *Arabidopsis*

Abstract

The sessile life of plant directed their evolution toward multiple adaptive strategies. Rapid protein turn over has been described to be a key regulatory mechanism for plant adaptation. Ubiquitin-modified proteins are targeted for degradation by the 26S-proteasome. A class of ubiquitin-ligases, the Cullin Ring Ligases (CRLs) have been shown to be involved in most *Arabidopsis* developmental processes. CRLs are stabilized by the covalent binding of the small peptide RELATED TO UBIQUITIN (RUB). The CRLs modification is required for the activity of plant hormones as exemplified by mutants deficient in the RUB-activating enzyme subunit AUXIN-RESISTANT 1 (AXR1). The perception of auxin results in the ubiquitin-mediated degradation of the AUXIN/INDOL-3-ACETIC ACID (Aux/IAA) transcriptional repressors. Aux/IAA proteins control the auxin-mediated transcriptional response. In this work, a forward chemical genomic strategy has been used to identify small synthetic molecules affecting plant development. We used the resistance of the *axr1-30* mutants in order to select compounds requiring RUB activation. Among the molecules isolated to alter specific plant developmental processes, three Developmental Regulators (DRs) have been shown to directly interfere with the degradation of the Aux/IAA proteins promoting a rapid induction of specific auxin-related transcriptional responses. Furthermore, we used the molecule DR4, abolishing specifically apical hook formation, to investigate the functional selectivity of auxin perception during apical hook development. A forward genetic screen has been performed to isolate *dr4-resistant* mutants. Several viable mutants were isolated with different sensitivity to auxin but all resistant to DR4. The isolation of mutants preferentially resistant to the differential growth defect induced by DR4 demonstrates the potential to determine the molecular process mediating the developmental features induced by the selective agonists of auxin. Since the first digital image 60 years ago, imaging techniques are constantly evolving generating more and more digital images. The conversion of images into biologically relevant quantitative data is an essential process to overcome and understand biological variability. In this work, we describe two digital images processing approaches which have been used to semi-automatically describe intracellular structure density and colocalization; the complex shape of the *Arabidopsis* pavement cells.

Keywords: Arabidopsis, chemical genomic, auxin, digital image processing

Author's address: Thomas Vain, SLU, Department of Forest Genetics and Plant Physiology, SE-901 83, Umeå, Sweden
E-mail: thomas.vain@slu.se

Dedication

To my family, my supervisors and everyone who supported me to make this thesis,

“The most fruitful basis of the discovery of a new drug is to start with an old drug.”

Sir James Whyte Black

Contents

List of Publications

This thesis is based on the work contained in the following manuscripts and papers, referred to by Roman numerals in the text:

I Vain T., Ferro N., Barange D.K., Kieffer M., Ma Q., Thelander M., Pařízková B., Novák O., Doyle S.M., Ismail A., Enquist P.A., Rigal A., Langowska M., Harborough S.R., Zhang Y., Ljung K., Callis J., Almqvist F., Kepinski S., Estelle M., Pauwels L. and Robert S.. Selective degradation of Aux/IAA proteins modulates plant development (manuscript).

II Vain T., Crowell E.F., Timpano H., Biot E., Desprez T., Mansoori N., Trindade L. M., Pagant S., Robert S., Höfte H., Gonneau M. and Vernhettes S. (2014). The Cellulase KORRIGAN Is Part of the Cellulose Synthase Complex. *Plant Physiology*. vol(165), 1521-1532.

III Majda M., Grones P., Sintorn I.M., Vain T., Milani P., Krupinski P., Zagórska-Marek B., Viotti C., Jönsson H., Mellerowicz E.J., Hamant O. and Robert S.. Mechano-chemical polarization of cell walls drives shape coordination (manuscript).

Papers II is reproduced with the permission of the publisher.

The contribution of Thomas Vain to the manuscripts and papers included in this thesis was as follows:

I Contributing to experiments, result analysis and writing the manuscript.

II Contributing to experiments, result analysis and summary of the results.

III Contributing to pavement cell shape quantification.

Abbreviations

All abbreviations are explained when they first appear in the text.

Introduction

1.1 *Arabidopsis thaliana*

The use of *Arabidopsis thaliana* (called *Arabidopsis* hereafter) was firstly reported in the early 20th century by Friedrich von Laibach (Laibach, 1907). In 1943, Laibach highlighted the interests of *Arabidopsis* as a model for genetics and developmental biology: a small size; easy to cultivate; 4 to 5 weeks generation time; a production of a large progeny; auto-fertilization; a low chromosome number; a large variation in physiological traits among subspecies and ecotypes (Laibach, 1943).

Arabidopsis became of real importance at the end of the 20th century with the rise of interest in molecular biology. The successive discoveries of the *tumor-inducing* plasmid in *Agrobacterium tumefaciens* (Larebeke *et al.*, 1975) opened the possibilities to perform horizontal and stable gene transfer in higher plants (Chilton *et al.*, 1977) and in particular in *Arabidopsis* (Lloyd *et al.*, 1986). *Arabidopsis* has one of the smallest genome among higher plants, which, associated to its diploid condition, allowed rapid positional cloning of natural or mutagenized variants in a population (Meyerowitz & Pruitt, 1985; Estelle & Somerville, 1986; Meyerowitz, 1987).

Arabidopsis genome was the second of all multicellular organism genomes to be sequenced, and the first genome available within the plant kingdom (The Arabidopsis Genome Initiative, 2000). Within the last century, *Arabidopsis* research has been rising exponentially as illustrated by more than 50,000 *Arabidopsis* publications in 2015 (Provart *et al.*, 2016). The improvement of analytical methods and the discovery of new molecular processes in *Arabidopsis* have led to significant breakthroughs in biology (Jones *et al.*, 2008). The "one genome = one specie" equation has moved forward with 1,135 *Arabidopsis* ecotypes sequenced to date (Alonso-Blanco *et al.*, 2016). This set of genomic data revealed the geographical distribution of genetic divergence and the potential to characterize the molecular basis of the plant capacity to

adapt to their environment (Gan *et al.*, 2011; Alonso-Blanco *et al.*, 2016). Genome-Wide Association Studies raised the possibility to associate phenotypes with natural mutations (Weigel, 2012).

1.2 Chemical genomics

1.2.1 Chemical genomics and library of synthetic organic compounds

The increasing knowledge in genetics, cell biology and biochemistry associated with a high throughput screening (HTS) of molecules radically changed the fields of pharmacology in the beginning of the 1990s. The pharmacological genetics (Mitchison, 1994), called today chemical genomics, aims to use small-molecules to alter a biological process via the modification of molecular functions, such as protein activity or protein conformation (Figure 1). The advantages of chemical genomics to classical genetics relay on the potential to analyse the rapid induction and reversion of the small-molecule effects (Robert *et al.*, 2009). Strategies to identify small molecules by a chemical genomic screening are analogous to genetic screening approaches: (i) the forward chemical genomics approaches to isolate active compounds based on a phenotype and without knowing their molecular targets and (ii) the reverse chemical genomics approaches to isolate compounds active on a defined molecular function (Stockwell, 2004; McCourt & Desveaux, 2010). Prior organic chemistry, chemical screenings were performed using natural resources and active compounds were enriched from natural extracts or natural products (Dayan *et al.*, 2009). Using synthetic compound libraries gave a direct access to the structure of the active molecules.

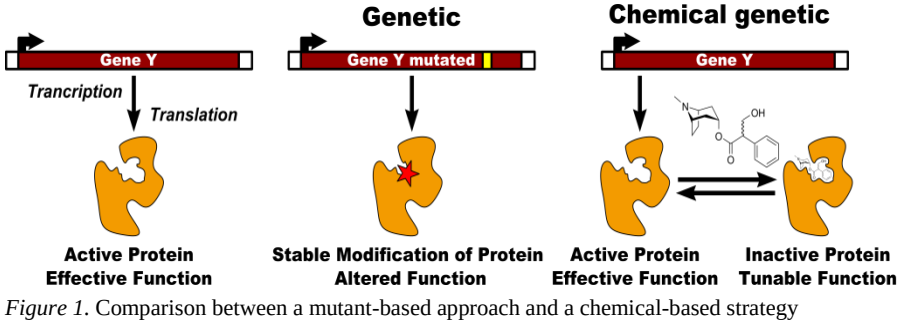

Figure 1. Comparison between a mutant-based approach and a chemical-based strategy

The development of combinatorial synthesis (synthesis from building blocks) opened the potential to screen either pure compounds or batches of synthetic compounds (Balkenhohl *et al.*, 1996). Since then, a tremendous amount of libraries with diverse characteristics has been developed. The

theoretical number of small-molecules with a molecular weight < 500 $g.mol^{-1}$ has been estimated to be higher than 10^{60} (Bohacek *et al.*, 1996; Dobson, 2004). To date (7th September 2016, 11:25 GMT), only 119,492,577 (10^8) pure organic and inorganic compounds have been registered by the Chemical Abstract Service (CAS). The difference in these numbers illustrates the potential of the chemical space to fulfil the need for novel chemical structures. Two kinds of chemical libraries are available: the biased libraries are generated founded on defined chemical structures (Tan, 2005), while the unbiased libraries contain the largest diversity of chemotypes (Gillet, 2008). Both kinds of libraries can contain fluorescent, polarized or tagged compounds (Ahn *et al.*, 2007; Simeonov *et al.*, 2008; Wagner *et al.*, 2008; Bachovchin *et al.*, 2014; Hall *et al.*, 2016). Literature and *in silico* screenings are now used to generate biased chemical libraries, which preferentially target specific protein functions, signalling pathways, developmental processes or poorly characterized enzyme activities (Lipinski & Hopkins, 2004; Lowrie *et al.*, 2004; Bachovchin *et al.*, 2009; Schreiber *et al.*, 2015). Since 2001, the possibility to perform HTS on library containing 1.5 million compounds in up to 1,536 well plates exists and opens many possibilities (Mayr & Bojanic, 2009).

The cost of chemical biology approaches is a strong limitation. Only initiative such as the National Institutes of Health (NIH) Molecular Libraries Initiative demonstrates the potential to isolate thousands of small molecules modulating specific cellular functions (Austin *et al.*, 2004; Schreiber *et al.*, 2015). Furthermore, the diversity achieved by combinatorial synthesis is still a limiting factor in comparison to the chemical complexity and diversity of small molecules present in biological systems. Thus, improving strategies for a natural product screening or the development of new libraries containing natural product-like molecules are current challenges in chemical biology (Dayan *et al.*, 2009).

1.2.2 Chemical genomics and *Arabidopsis* development

The research for organic compounds affecting plant development started in the beginning of the 20th century with the indol-3-acetic acid (IAA) presenting herbicide properties affecting preferentially the growth of the dicotyledons (Templeman & Marmoy, 1940). At the same time, growth substances such as colchicine and 1-naphtalene acetic acid (1-NAA) were described for their effects on the root meristem development (Borgstrom, 1939; Levan, 1939). Moreover, the research on herbicides resulted in the description of 2,4-dichlorophenoxy acetic acid (2,4-D) and 2,4,5-trichlorophenoxy acetic acid (2,4,5-T) synthesis (Pokorny, 1941). The design of libraries and the isolation of small molecules affecting plant growth are mostly accomplished by

agrochemical companies (Lamberth *et al.*, 2013; Wassermann *et al.*, 2014; Jeschke, 2016). It is worth to note that compounds active as herbicides have raised ethical, health and environmental concerns after being largely used in agriculture (Mithila *et al.*, 2011) or as biological weapons during several wars (Jacob & Walters, 2005).

Nevertheless, the use of small molecules on *Arabidopsis* contributed largely to characterize several pathways mediating activities of endogenous small molecules. For example, 2,4-D has been used in the first auxin-resistant screen in *Arabidopsis* and the identification of the *P83* mutant with an altered root growth orientation demonstrated that endogenous auxin participates in the response to gravity (Maher & Martindale, 1980). *P83* has a mutation in the *AUXIN RESISTANT 1* (*AUX1*) gene expressing a major auxin influx carrier demonstrating the importance of auxin transport for a gravitropic response (Bennett *et al.*, 1996; Marchant *et al.*, 1999). Another screen for 2,4-D resistant mutants led to the identification of 12 *auxin-resistant 1* (*axr1*) alleles (Estelle & Somerville, 1987). Among those, novel components were isolated, *AUX1*, *AXR1*, *AXR2* (Lincoln *et al.*, 1990; Wilson *et al.*, 1990), *AXR3* (Leyser *et al.*, 1996), *AXR4* (Hobbie & Estelle, 1995), *AXR5* (Yang *et al.*, 2004) and *AXR6* (Hobbie *et al.*, 2000). Additionally, *aux1*, *axr1* and *axr2* mutants were shown to be less affected by other hormones such as ethylene, cytokinin (CK) or abscisic acid (ABA) (Pickett *et al.*, 1990; Wilson *et al.*, 1990; Leyser *et al.*, 1993). This illustrates perfectly that approaches using small synthetic molecules have the potential to isolate key molecular components regulating plant development. Moreover, synthetic auxins have been shown to display selective properties. An example would be the picloram compound, which preferentially binds to one of the auxin receptors, the AUXIN F-BOX 5 (AFB5) (Walsh *et al.*, 2006; Calderón Villalobos *et al.*, 2012). Several approaches to understand selectivity among auxin synthetic analogues have been recently documented (Simon *et al.*, 2013; Lee *et al.*, 2014).

Chemical genomics screens in plants firstly aimed for an inhibitor or an enhancer of a known defect. An unbiased library was successfully screened to isolate inhibitors of a transcriptional auxin response (Armstrong *et al.*, 2004). Compounds enhancing or inhibiting root response to gravity were characterized and led to establish a direct link between gravitropism and the endomembrane system (Surpin *et al.*, 2005). Among those, several complex molecules with an auxinic moiety and biologically active as auxins suggested the potential to isolate auxin activated after hydrolysis, called pro-auxin (Christian *et al.*, 2008). Similarly, Savaldi-Goldstein and co-authors isolated a compound with a pro-auxin activity with the capacity to suppress the short

hypocotyl phenotype of *deetiolated2-1* (*det2-1*), a mutant deficient in brassinosteroids (BR) biosynthesis (Savaldi-Goldstein *et al.*, 2008).

The phenocopy-based screen was another strategy used to find small molecules inducing defects similar to those mediated by the signalling pathway of interest. This strategy led to the isolation of the structurally unique compounds, bikinin and naxillin for their potential to mimic brassinosteroid treatment or auxin induction of lateral root respectively (De Rybel *et al.*, 2009, 2012). Bikinin was characterized to simultaneously inhibit the activity of seven glycogen synthase kinase 3 (GSK3) proteins which are part of brassinosteroid-related signalling pathway (De Rybel et al. 2009). The characterisation of naxillin activity raised the importance of the indole-3-butyric acid conversion to IAA from the in the root cap to control root architecture (De Rybel *et al.*, 2012; Xuan *et al.*, 2015).

Chemical genomics strategies also contributed to the discovery of a novel family of endogenous small molecule receptors. The germination inhibitor, called pyrabactin, is a selective agonist of ABA and led to the identification of PYRABACTIN RESISTANCE 1/PYR1-LIKE (PYR1/PYLs) proteins, thus characterized as the ABA receptors (Park *et al.*, 2009). The selection of a PYR1 protein with a high affinity to the commercially available agrochemical mandipropamid opens up new strategies for the chemical control of plant adaptation to dry conditions (Mosquna *et al.*, 2011; Park *et al.*, 2015).

Thus, the advances made since small molecules have been applied to alter *Arabidopsis* development are illustrating the potential of chemical genomics to dissect complex signalling pathways.

1.2.3 Chemical genomics and endomembrane trafficking

Chemical genomic approaches have been relevant in studying the intracellular trafficking (Surpin & Raikhel, 2004). Interestingly, screens performed in *Saccharomyces cerevisiae* led to the discovery of compounds active in plants thanks to the conservation of some mechanisms regulating the endomembrane system between kingdoms (Zouhar *et al.*, 2004). Automated-microscopy and fluorescent markers for individual intracellular compartments have been at the basis of a chemical genomic screen which aimed to directly isolate compounds effective on plant intracellular integrity (Robert *et al.*, 2008; Drakakaki *et al.*, 2011). In order to efficiently isolate compounds targeting the endomembrane system, a library of natural products was screened for altered pollen germination, a developmental process involving an active endomembrane trafficking to control the pollen tube tip growth (Robert *et al.*, 2008). Within the isolated compounds, referred to as endosidins, some led to the mislocalization of a fluorescent marker normally localised to the tip of the

pollen tube (Robert *et al.*, 2008). Among them, endosidin1 selectively affects the cycling of certain plasma membrane proteins (Robert *et al.*, 2008), by stabilizing actin microfilaments (Tóth *et al.*, 2012). This approach identified many compounds with the potential to alter more or less selectively the endomembrane integrity (Drakakaki *et al.*, 2011). Several endosidin molecules have been further characterized to affect different trafficking routes and for more details, please refer to Mishev *et al.*, 2013, Doyle *et al.*, 2015 and Klíma *et al.*, 2015.

1.2.4 Target identification in forward chemical biology

Forward chemical genomic screens aim to isolate molecules altering a biological process of interest. This chemical screening approach leads to two questions: (i) Is the molecule of interest known? (ii) How to identify the target of the molecule? The first question can be answered by using structure similarity search in database such as Pubchem (Kim *et al.*, 2015). Answering the second question is more complex. Genetic screen to identify resistant mutants has shown great results especially if the molecules are selective (Park *et al.*, 2009) or inducing similar defect as endogenous compounds (De Rybel *et al.*, 2012). Moreover, several target identification strategies have been developed with or without structural modification of the small molecules (Lee & Bogyo, 2013; Schenone *et al.*, 2013; Tresch, 2013; Ziegler *et al.*, 2013; Dejonghe & Russinova, 2014):

- Target isolation by affinity purification:
    - Synthesis and selection of tagged active analogues
    - Photo-Cross linking approaches (Kanoh, 2016)
- Chemical proteomics (chemoproteomics):
    - Drug-Affinity Responsive Target Stability (DARTS)
    - Stability of Protein from Rates of Oxidation (SPROX)
    - Activity-Based Protein Profiling (ABPP)
- Expression cloning techniques:
    - Yeast-3-hybrid
    - mRNA display
    - Phage display

After the identification of the target, the mechanism of action of the molecule could be investigated. A chemical genomic screen could end with the isolation of rare allosteric inhibitors but also with compounds altering a protein function in competition with the endogenous ligand. Several experiments are required to estimate binding affinity, such as surface plasmon resonance (SPR), *in vitro* pull-down or Isothermal Titration Calorimetry (ITC). One should take into account that endogenous molecules are often having affinity for the

binding pocket of several proteins. ABPP have been used in combination with unspecific antagonists to characterize more precisely the potential selectivity of compounds within protein families or within the full proteome (Niphakis & Cravatt, 2014). The small-molecules need to be engineered to introduce several structural modifications in order to create probes. Combinatorial synthesis focused on generating probe-candidate library enabling the feasibility to rapidly isolate structurally diverse probe-candidates for further characterization of the compound mechanism of action (Yang & Liu, 2015).

1.3 Auxin

1.3.1 Auxin metabolism

During the end of the 19th century, Julius von Sachs suggested the existence of moving substances coordinating plant growth (Sachs, 1890). At the same time, Charles Darwin and his son Francis demonstrated the requirement of a transported signal to modulate plant growth (Darwin & Darwin, 1881). Auxin chemical structure has been characterized as being indole acetic acid in 1934 by Kögl's and Thimann's labs (Kögl *et al.*, 1934; Wildman, 1997). Since then, several endogenous molecules were identified as auxinic compounds (Simon & Petrášek, 2011; Enders & Strader, 2015). The synthesis of IAA is mainly initiated from L-Tryptophan (Trp) through the indole-3-pyruvic acid (IPyA) pathway (Kasahara, 2015). Nevertheless, IAA has been shown to be also synthetized by other Trp-dependent or –independent metabolic pathways (Ljung, 2013; Kasahara, 2015). Beside the production of auxin, its catabolism is a key player in the regulation of auxin homeostasis. This step can occur by (i) a reversible or irreversible conjugation of IAA to amino acid or sugar, or by (ii) IAA-oxidation and degradation (Woodward & Bartel, 2005; Ljung, 2013; Rampey *et al.*, 2013; Mellor *et al.*, 2016; Porco *et al.*, 2016). Interestingly, 2-oxoindole-3 acetic acid (oxIAA), a IAA catabolite, has been shown to be a weak IAA-like compound on plant development and to be perceived by the nuclear auxin signalling machinery *in vitro* (Pencík *et al.*, 2013).

Recent progresses of chemical genomics and structure-based rational design have been used to characterize many compounds with an auxin-like activity or auxin-related activity (Ma & Robert, 2014). Compounds such as L-kynurenin, L-amino-oxyphenylpropionic acid (L-AOPP) and yucasin, all inhibitors of enzymes in the IPyA pathway, confirmed the results obtained using genetic approaches as the compounds were mimicking auxin-deficient mutants and reverting IAA-overproducing mutants (Kasahara, 2015). The isolation of new compounds affecting auxin metabolism is essential to develop pharmacological approaches that will functionally challenge the redundancy of the regulatory

mechanism governing auxin homeostasis during plant development. Finally, the use of synthetic auxin analogues confronted to similar enzymatic conversion than endogenous auxin could facilitate the traceability of auxin-like molecule conversion in plant (Eyer *et al.*, 2016).

1.3.2 Auxin transport

The interest in characterizing the nature of moving signal in plants raised when such signal from young to old cells had been suggested to mediate coleoptile phototropism (Darwin & Darwin, 1881). It has been shown that the protonated IAA can diffuse better through the plasma membrane compared to the deprotonated form (Raven, 1975). The pH difference between symplast and apoplast leads to a passive diffusion of IAAH within the symplast, where it is deprotonated into IAA^- and gets concentrated (Rubery & Sheldrake, 1974). However, deprotonated auxin is also actively transported through the plasma membrane via auxin carriers. Auxin -influx and -efflux facilitators contribute to the formation of an auxin gradient, essential for most of the plant developmental processes (Friml, 2003; Petrásek & Friml, 2009). Several carriers have been described with intracellular and tissue specific localization:

- PIN-FORMED [PIN] family are transmembrane proteins carrying IAA from the cytoplasm to the apoplast or to/from the endoplasmic reticulum lumen depending of their subcellular localization (Adamowski & Friml, 2015). Plasma membrane localised PIN proteins are asymmetrically distributed in the cell (Gälweiler *et al.*, 1998; Müller *et al.*, 1998; Friml *et al.*, 2002a, 2002b).
- PIN-LIKEs [PILs] are transmembrane proteins localized at the endoplasmic reticulum. They are suggested to contribute to intracellular auxin accumulation (Barbez *et al.*, 2012).
- AUXIN1 [AUX1] / LIKE-AUX1 [LAXs] are plasma membrane permeases carrying auxin from the apoplast to the cytoplasm (Bennett *et al.*, 1996; Swarup *et al.*, 2008; Péret *et al.*, 2012).
- Some ABCB / P-GLYCOPROTEIN [PGP] transporters have been shown to export auxin from the cytoplasm to the apoplast (Noh *et al.*, 2001; Geisler *et al.*, 2005).
- Some NITRATE TRANSPORTER1 / PEPTIDE TRANSPORTER Family (NPF) have been shown to transport IAA (Krouk *et al.*, 2010; Léran *et al.*, 2014).

The various levels regulating auxin transporter activities are complex and mediated in response to various environmental and endogenous stimuli at the transcriptional and post-transcriptional levels (Luschnig & Vert, 2014; Doyle *et al.*, 2015; Willige & Chory, 2015; Armengot *et al.*, 2016). Furthermore,

auxin signalling and induced transcriptional response have been shown to play a critical role in a feedback loop regulating auxin flux (Schrader *et al.*, 2003; Vieten *et al.*, 2005; Sauer *et al.*, 2006). Small-molecules such as 1-naphthylphthalamic acid (NPA), an auxin efflux inhibitor, have been used to study the importance of auxin efflux during various developmental processes (Klíma *et al.*, 2015). The isolation of NPA resistant mutants led to the identification of the *TRANSPORT INHIBITOR RESPONSE1* (*TIR1*) gene (Ruegger et al. 1997; Ruegger et al. 1998) which have been characterized as a member the auxin receptor gene family (Dharmasiri *et al.*, 2005; Kepinski & Leyser, 2005). NPA has been shown to target auxin efflux carrier distribution by altering their association with the actin cytoskeleton (Geisler *et al.*, 2003; Zhu *et al.*, 2016).

The structure-based synthesis of auxin-related molecules brought several alkoxy-auxins acting as a new class of auxin transport inhibitors (Tsuda *et al.*, 2011). Auxin transporter inhibitors have also been isolated by chemical genomic screen such as the isolation of BUM (2-[4-(diethylamino)-2-hydroxybenzoyl]benzoic acid), which compared to NPA displays more specificity to inhibit auxin efflux through ABCB proteins rather than PIN proteins (Kim *et al.*, 2010). Thus, genetic and pharmacological approaches are complementary to characterize the key players mediating auxin transport in particular developing organs.

1.3.3 Auxin perception and transcriptional response to auxin

The complexity and the combinatorial properties of auxin signalling components revealed another level of complexity mediating auxin pleiotropic effects (Ulmasov *et al.*, 1997; Vernoux *et al.*, 2011; Calderón Villalobos *et al.*, 2012; Boer *et al.*, 2014; Salehin *et al.*, 2015; Weijers & Wagner, 2016). The expression level of each auxin responsive gene is regulated by the auxin concentration and the affinity between components of the signalling pathway (Weijers & Wagner, 2016). Auxin is perceived in the nucleus by the TIR1/AFB$^{1-5}$-AUXIN/INDOL-3-ACETIC ACID (Aux/IAA) co-receptor complex (Calderon-Villalobos *et al.*, 2010; Salehin *et al.*, 2015). The TIR1/AFB1-5 F-BOX proteins act as auxin receptors (Dharmasiri *et al.*, 2005; Kepinski & Leyser, 2005; Walsh *et al.*, 2006; Parry *et al.*, 2009; Hu *et al.*, 2012; Prigge *et al.*, 2016). The formation of the TIR1/AFBs-Aux/IAA complex leads to the ubiquitination of the Aux/IAA transcriptional repressors targeting them for rapid degradation by the 26S-proteasome (Worley *et al.*, 2000; Zenser *et al.*, 2001; Maraschin *et al.*, 2009). The rate of Aux/IAA degradation in the presence of IAA differs among the 29 Aux/IAAs (Dreher *et al.*, 2006; Havens *et al.*, 2012). This variation is dependent on the amino acid identity within the

DII domain of the Aux/IAA proteins which are essential for the formation of the co-receptor (Dreher *et al.*, 2006; Calderón Villalobos *et al.*, 2012). Nevertheless, residues outside the DII domain also contribute to the Aux/IAA stability and their degradation rate (Moss *et al.*, 2015). The multiplicity of potential co-receptor assembly associated to their different sensitivities to IAA is the first element controlling the specificity of the auxin response. Indeed, mutation altering the potential of the Aux/IAA to be degraded leads to pleiotropic developmental defect as well as strong auxin insensitivity (Reed, 2001; Lavy *et al.*, 2016).

An additional level of complexity is governed by the strength of the protein interaction between the Aux/IAA and the AUXIN RESPONSE FACTORS (ARFs) (Tiwari *et al.*, 2004; Vernoux *et al.*, 2011; Piya *et al.*, 2014). Specific Aux/IAA-ARF combination are required to modulate precise developmental processes (Weijers *et al.*, 2005; Smet *et al.*, 2010; Yamaguchi *et al.*, 2013). The co-evolution of amino acid sequences responsible for the Aux/IAA- ARF interaction revealed the essentiality of these interactions for plant development (Paponov *et al.*, 2009).

In absence of auxin, Aux/IAA proteins interact with ARF transcription factors and recruit the co-repressor protein TOPLESS (Szemenyei *et al.*, 2008). Interestingly, TOPLESS has been shown to recruit the HISTONE DEACETYLASE 19 (HDA19), which removes the histone acetylation leading to a condensed DNA structure (Krogan *et al.*, 2012). In presence of auxin, the Aux/IAA proteins are degraded and the ARF protein MONOPTEROS/ARF5 has been shown to physically interact with two chromatin remodelling proteins BRAHMA (BRM) or SPLAYED (SYD) in order to repress condensed chromatin state at the ARF5 locus (Wu *et al.*, 2015). The ARF-DNA binding locus, called AuxRE, are TGTCTC (Ulmasov *et al.*, 1995, 1997) and TGTCGG (Boer *et al.*, 2014). The capacity of the ARFs to regulate gene expression also involves their potential to form dimers that bind to auxin response DNA elements (AuxRE) (Boer *et al.*, 2014). AuxRE are highly present in the genome and are often associated with other *cis* elements such as Y-patch or ABRE to finely regulate auxin-mediated gene transcription (Mironova *et al.*, 2014; Zemlyanskaya *et al.*, 2016).

1.4 Rubbylation of CRLs controls auxin sensitivity

Recent progresses towards a better understanding of the molecular mechanisms of hormone signalling have revealed the importance of modulating the protein level for switching on and off signalling pathways via the ubiquitin proteasome system (UPS) (Santner & Estelle, 2010; Kelley & Estelle, 2012). Remarkably,

this mechanism is highly evolutionary conserved. Various post-translational modifications, such as the binding of small polypeptide modifiers, appear to play an essential role in the regulation of protein stability (Downes & Vierstra, 2005; Hochstrasser, 2009). In the early 1980s it was found that ubiquitous (in animals and plants) peptides such as ubiquitin (Ub), when covalently attached to a protein, generate a signal recognized by a large protein-degrading enzyme complex, the 26S proteasome (Pickart & Cohen, 2004). The linkage of Ub requires several enzymes and occurs in three steps: the Ub is first activated by an Ub-activating enzyme (E1), the activated Ub is then passed to an Ub-conjugating enzyme (E2), which works in concert with an Ub-protein-ligase (E3) to link the Ub to the targeted protein and send it for degradation (Kerscher *et al.*, 2006). Other small peptide modifiers have been discovered, such as RELATED-TO-UBIQUITIN/NEURAL PRECURSOR CELL EXPRESSED DEVELOPMENTALLY DOWNREGULATED PROTEIN 8 (RUB/NEDD8), which require the same E1, E2, E3 enzymatic cascade to be covalently linked to the RUB-modified proteins (Hotton & Callis, 2008; Mergner & Schwechheimer, 2014; Enchev *et al.*, 2015). Some E3 Ub-ligases are part of a multimeric protein complex categorized in at least three classes of CULLIN RING LIGASE (CRLs) in plants (Hua & Vierstra, 2011). CRLs are composed of a scaffold proteins called CULLIN (CUL) which are RUB-modified (Pozo *et al.*, 1998; del Pozo & Estelle, 1999; Petroski & Deshaies, 2005; Hotton & Callis, 2008; Sarikas *et al.*, 2011).

Plant studies have made key contributions in elucidating the UPS-mediated signalling processes, as the first RUB-activating enzyme was discovered in *Arabidopsis* (Leyser *et al.*, 1993). Further studies have shown that more than 5% of the proteins in *Arabidopsis* participate in the UPS (Vierstra, 2003). Interestingly, more than 1,500 genes encode E3s and these include almost 900 encoding F-BOX proteins, compared to 20, 27 and 69 in *Saccharomyces cerevisiae*, *Drosophila melanogaster* and the human genome, respectively (Skaar & Pagano, 2009). Loss of any of the components reducing RUB-conjugation, such as the activity of the RUB-activating enzyme AXR1, leads to a hormone-related phenotype, including resistance to auxin, cytokinin, ethylene, brassinosteroid and jasmonate (Leyser *et al.*, 1993; Timpte *et al.*, 1995; Schwechheimer *et al.*, 2001; Tiryaki & Staswick, 2002). RUB-modification of CRLs is essential to mediate plant sensitivity to endogenous and environmental stimuli (Hotton & Callis, 2008; Kelley & Estelle, 2012). Auxin is perceived by one type of CRL where TIR1/AFBs proteins are the F-BOX subunits of the S-PHASE KINASE ASSOCIATED PROTEIN 1/CULLIN 1/F-BOX (SCF) Ub ligase. In presence of auxin, TIR1/AFB binds to the Aux/IAA proteins that will be poly-ubiquitinated after recruitment of

Ub-conjugating enzymes such as UBC13 (Del Pozo & Manzano, 2014; Wen *et al.*, 2014).

1.5 Digital image processing

1.5.1 Digital image and basics of image restoration

A digital image corresponds to data (pixels or voxels) arranged in a dot matrix. The first digital image was a scanned picture in 1957 by Russell A. Kirsch using the Standards Eastern Automatic Computer (Kirsch *et al.*, 1958). Since then, all types of cameras have been equipped with sensors transforming light into discrete signal. Digital image processing aims to remove the noise generated by the sensor (restoration) and extract quantitative value based on the distribution of the discrete signal in the dot matrix (Takeda *et al.*, 2007). All the following illustrative examples and processing have been made using the ImageJ public domain software (https://imagej.nih.gov/ij/index.html). Pre-processing or image restoration increases the signal/noise ratio by removing the called noise generated in the digital image by the sensor. Several complex algorithms have been developed in order to perform image restoration (Boulanger *et al.*, 2007). Image processing can often be impossible without image pre-processing.

1.5.2 Image segmentation and shape characterization

A scaled digital image of a sample affords the possibility to extract automatically many information such as sample perimeter, surface, angle and length. In order to access this information, the images must be converted into binary signal to discriminate the signal of interest and its geometry within the dot-matrix (Figure 2a). Binary pictures can be obtained by various methods such as defining a threshold to discriminate between background and sample discrete value. The shape descriptors are calculated to characterize (i) the perimeter and area of the structure (Figure 2b); (ii) the circularity which measures the form and the roughness of the shape (Figure 2c); (iii) the roundness which measures the ratio between an equivalent diameter, calculated from the area of the structure, with the maximal length of the structure (Figure 2d). Both circularity and roundness coefficients are approaching a value of one for spherical structures. However, circularity coefficient is more sensitive than roundness to small-scale bumps that increase the perimeter. The quantitative data can then be used to compare shape variability within or between different samples, without the bias of manual measurement or visual appreciation.

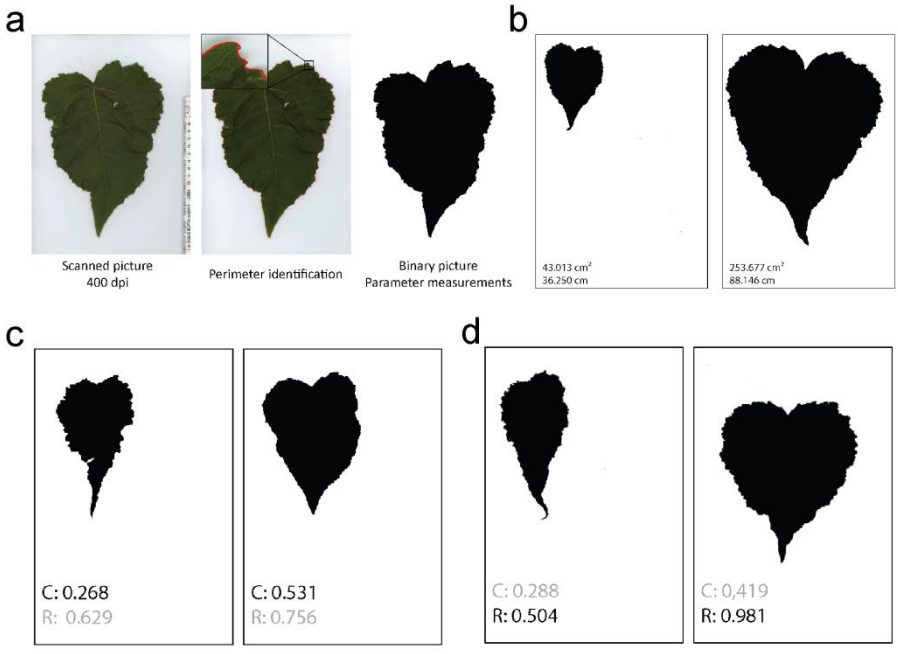

Figure 2. Scanned image segmentation and shape characterization. (a) Scanned picture processed by binary transformation after isolating the edge of the sample (red line) and holes filled within the structure. (b) Discrimination of structure according to the surface or perimeter. (c) Discrimination of structures according to circularity (C) with almost similar roundness (R). (d) Discrimination of structures according to the roundness with almost circularity.

1.5.3 TopHat transformation for subcellular structure colocalization

The TopHat transformation consists in removing an opened image from the pre-proceeded image (Serra, 1982). The opened image is obtained by the combination of two morphological operators: the erosion and the dilatation. *A priori* knowledge about the shape of the studied structure (e.g. vesicles in Figure 3) is needed to determine the use of an appropriated shape for the two operators. This shape is named structuring element (SE). The erosion shrinks all the structures in the image, whose shape and size are inferior or equal to the one defined for the SE (Figure 3). In this case we were interested in extracting a circle with an average diameter of seven pixels. After TopHat transformation, Pearson's correlation coefficient (PC) was computed to estimate the correlation of the signal intensity on each overlapping pixels between both channels of the dual-channel image (Bolte & Cordelières, 2006). The thresholds have been set according to the signal of interest. The result of this segmentation is a binary picture to discriminate pixels with a signal and pixels without a signal. The overlap between pixels with signal was analysed using the Manders coefficient (Manders *et al.*, 1993). The Manders coefficients are computed to estimate the

fraction of overlapping pixels in each channel (Figure 3c). Pearson's and Manders's coefficients are used to estimate the co-localizing fraction of each marker (Bolte & Cordelières, 2006).

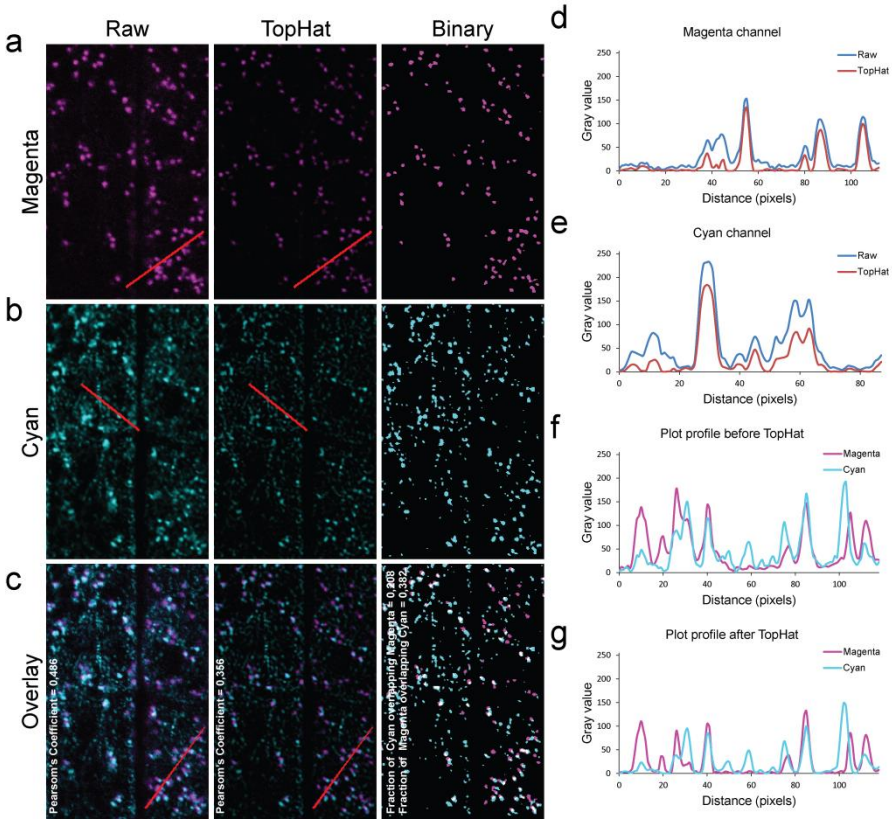

Figure 3. TopHat transformation of a dual channels image of endosomes for colocalization quantification. Raw images processed using TopHat transformation and binary pictures were obtained after applying a grey value-based threshold on TopHat transformed pictures for (a) the magenta channel and (b) the cyan channel. (c) Overlay of the magenta and cyan channels for each processing step. (d-e) Plot profile of the red line in (a) and (b) representing the effect of TopHat transformation on (d) the magenta signal and (e) the cyan signal respectively. (f-g) Plot profile representing the signal overlay along the red line in (c) before (f) and after (g) TopHat transformation.

2 Objectives

- Isolate, characterize and use molecules active on plant development through the AXR1-mediated signalling pathway

- Collaboration to develop and apply digital image processing workflow

3 Results and Discussion

3.1 Chemical genomics and isolation of selective agonists of auxin (Paper I)

3.1.1 Forward developmental defect-based chemical genomic screen

We performed a forward chemical genomic screen to identify compounds with an AXR1-dependent activity. We screened 8,000 synthetic small molecules for altered development of the wild-type (Col-0) *Arabidopsis* plants with the *axr1-30* mutant plants as a control (Hotton *et al.*, 2011). Thirty-four compounds were identified affecting either hypocotyl and/or primary root length. Including *axr1-30* as a secondary screen performed in parallel, led to the direct isolation of compounds acting through the AXR1-mediated signalling pathway.

As auxin has an important role in the development of *Arabidopsis* seedlings, we used transgenic plants expressing β-glucuronidase as a reporter under the synthetic auxin responsive promoter p*DR5*::GUS (Ulmasov *et al.*, 1997) to test the potential of the compounds to induce an auxin response. Seventeen compounds (out of 34, 50%) were inducing p*DR5*::GUS in the root tip after 5h treatments.

3.1.2 Tanglegram as a Structure Activity Relationship (SAR) representation

The structure similarity of the 34 isolated compounds was assessed using structure similarity clustering using ChemMine (Backman *et al.*, 2011). The 34 compounds were clustered in three major groups and most of them contained at least two carbon rings. Some compounds display an auxinic substructure such as a 2,4-D or a naphthalene moiety. We have identified six classes of compound-induced phenotypes. Interestingly, similar phenotypes were induced by structurally different compounds while structurally similar compounds were inducing diverse phenotypes. The phenotypes induced by the identified compounds were independently clustered. The two independent hierarchical clusters were linked with auxiliary lines in a tanglegram which has been

untangled using Treemap3 (Charleston & Robertson, 2002). Tanglegram is a recent approach used in phylogeny and co-evolutionary analysis (Scornavacca *et al.*, 2011; Matsen *et al.*, 2015). The tanglegram representation revealed that two group of structurally similar molecules were not inducing an auxin response and had distinct activities on development. The four most potent compounds, called Developmental Regulators (DR1-4), were chosen within these two groups of molecules.

3.1.3 Reverse chemical genetics to identify the chemically targeted signalling pathway

AXR1 regulates the activity of three types of CRLs in plants (Santner & Estelle, 2010). The compounds were assayed for their activity on mutants of each type of CULLIN. All the compounds were less active on *cul1-6* (Moon *et al.*, 2007), showing that CUL1 is required for the activity of the DRs. Moreover, these results also demonstrate that the DR compounds were not acting on a general mechanism regulating CRLs activity. CUL1, backbone of the SCF complex, is an essential component of the auxin signalling pathway. Thus, we assayed the requirement of the auxin perception machinery for the activity of the DRs. We demonstrated the requirement of the auxin receptors by showing the less sensitivity of *tir1-1* (Ruegger et al. 1998) and *tir1-1afb1-3afb3-4* mutants to the DRs effects. Furthermore, auxinole, an auxin antagonist (Hayashi *et al.*, 2012), used in co-treatment with the DRs, was reducing their activity validating the requirement of a proper degradation of the Aux/IAA proteins for the DR effects.

3.1.4 Selective agonists

Some close analogues of the DR molecules have been described as pro-drugs or pro-auxins (Christian *et al.*, 2008; Savaldi-Goldstein *et al.*, 2008). A pro-drug is inactive, but more permeable that the initial drug and the active drug is released after hydrolysis in specific tissues (Rautio *et al.*, 2008). In order to test the potential pro-drug activity of the DRs, we assayed their purity and stability. We showed that the DRs were not stable and are metabolized into potent auxin agonists, detected in the media and plant tissues. Interestingly, the presence of plants in the media increased the degradation product concentration in the media suggesting an enzymatic conversion. In order to identify if we could correlate a tissue specific auxin response with the induced phenotypes, we used several lines expressing transcriptional reporter fusions described to be responsive to auxin. All the tested reporter lines were reproducibly induced by auxin. DR1, DR3 and DR4 were able to promote the expression of the reporter in at least one of the lines tested in tissues where IAA was also inducing auxin

response. These data suggested that only a part of the transcriptional auxin response was induced by the DRs and not in a tissue dependent manner. In order to confirm this hypothesis, a transcriptomic profiling of cell culture after a short treatment with IAA, DR3 and DR4 was performed. Analysis of the up-regulated genes revealed that these two DRs were inducing independently some IAA-dependent gene clusters. Thus, we hypothesized that the DRs might be directly and selectively perceived by the auxin perception machinery.

The crystal structure of the auxin co-receptor complex (TIR1-IAA-Aux/IAA7 degron) is available on the RCSB Protein Data Bank (www.pdb.org) and referred as 2P1Q (Tan *et al.*, 2007). Thus, we performed docking experiment in the 2P1Q structure using the webserver SwissDock (Grosdidier *et al.*, 2011) and the DR-structures ready-to-dock in the ZINC[12] database (Irwin *et al.*, 2012). The best docking results demonstrated that the four DRs were able to physically fit within the auxin binding pocket. Quantum computations were performed to compare the potential of the DRs to thermodynamically stabilize TIR1 as was shown for the IAA. We showed that apart from DR2, the conformations that were able to stabilize TIR1 were close to the conformation of the molecules when docked in the co-receptor complex. Then, we performed *in vitro* pull down to test the capability of the DRs to promote the formation of the co-receptor complex (Kepinski, 2009). IAA was increasing TIR1 recovery with every Aux/IAA proteins tested. Interestingly, depending of the Aux/IAA proteins used to pull-down TIR1, the DRs were able to promote with different strength the recovery of TIR1. The degradation of various Aux/IAA proteins was assayed *in planta* and in presence of the DRs using a luciferase assay (Gilkerson *et al.*, 2009). The DRs were able to selectively promote the degradation of some Aux/IAA while IAA was effective on the four different constructs tested. Overall, our data demonstrated that three DRs act as selective agonists of auxin to modulate plant development.

3.2 Forward genetic screen to isolate DR4 resistant mutants

We showed that upon DR4 treatment, *Arabidopsis* plants grown in light have a longer hypocotyl and present a defect in phototropism at high concentration (paper I). Nevertheless, DR4 treatment applied on *Arabidopsis* grown in dark abolished the apical hook formation and reduced the hypocotyl length only at higher concentration (Figure 5 a-d). The lower sensitivity of *axr1-30*, *cul1-6* and *tir1-1afb1-3afb3-4* mutants demonstrated the requirement of an activated and functional auxin perception complex to mediate DR4 induced apical hook opening in the dark (Figure 5c). These mutants were also less affected by the DR4 effects on hypocotyl length suggesting that a functional auxin perception

machinery was also required for the DR4-induced inhibition of hypocotyl elongation (Figure 5d). To identify molecular actors mediating the DR4 effects, we performed a DR4 resistant screen on a M2 EMS population of Col-0. Ten thousands chemically mutated seedlings have been screened for a hook back phenotype in presence of DR4.

Within the isolated mutants, three independent lines carrying a recessive mutation conferring resistance to the DR4 effect in light have been selected (*dr4-resistant* mutants; paper I). Under light growth conditions, the mutations conferring DR4-resistance were associated with different levels of sensitivity to treatments with IAA, 2,4-D or other DRs. These mutants demonstrated the potential to isolate mutations associated with the auxin response selectively induced by DR4. Moreover, the short root phenotype of two *dr4-resistant* mutants (*dr4-r1* and *dr4-r3*) and their sensitivity to IAA and 2,4-D demonstrate that those mutants would have been missed using a forward genetic screen to identify IAA or 2,4-D resistant mutants.

Among the isolated mutants, five of them were considered for further analyses of their phenotypes in presence or absence of DR4 (Figure 5e). The *hookback* mutations were co-segregating with effects on various developmental processes requiring regulated differential growth such as apical hook maintenance (Figure 5f), leaf shape and serration (Figure 5g). The identification of the mutated nucleotides and functional characterization of the mutated genes will bring new insights into the auxin-mediated differential growth.

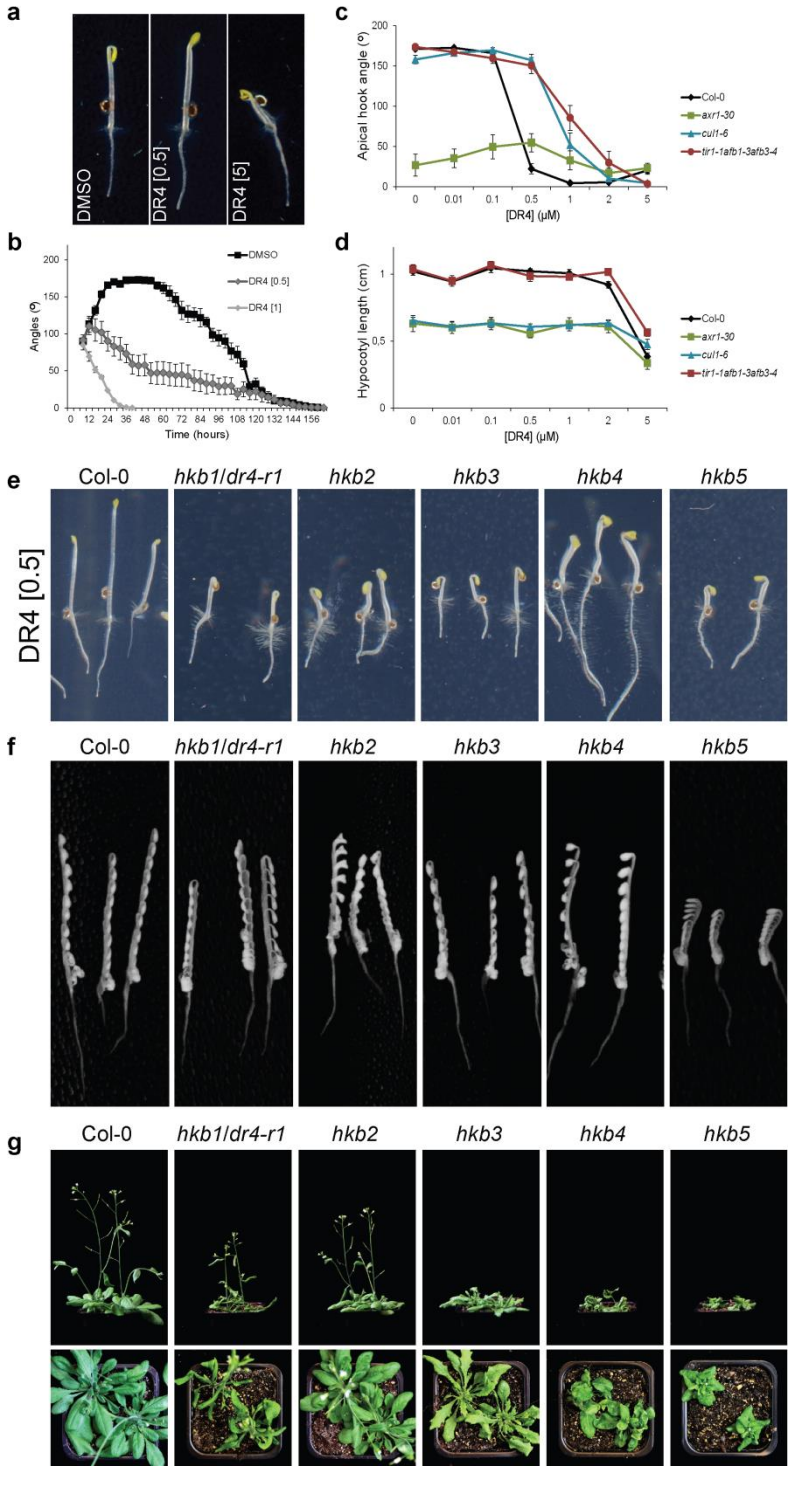
a
DMSO
DR4 [0.5]
DR4 [5]
b
DMSO
DR4 [0.5]
DR4 [1]
Angles (°)
Time (hours)
c
Apical hook angle (°)
[DR4] (µM)
Col-0
axr1-30
cul1-6
tir1-1afb1-3afb3-4
d
Hypocotyl length (cm)
[DR4] (µM)
Col-0
axr1-30
cul1-6
tir1-1afb1-3afb3-4
e
DR4 [0.5]
Col-0
hkb1/dr4-r1
hkb2
hkb3
hkb4
hkb5
f
Col-0
hkb1/dr4-r1
hkb2
hkb3
hkb4
hkb5
g
Col-0
hkb1/dr4-r1
hkb2
hkb3
hkb4
hkb5

Figure 4. DR4 abolishes apical hook formation and *dr4-resistant* mutants (*dr4-r*; *hookback*; *hkb*). (**a**) Three day-old Col-0 seedlings grown on two concentrations of DR4. (**b**) Apical hook angle of Col-0 measured every four hours after germination in DMSO or in presence of DR4. DR4 affects apical hook angle (**c**) and hypocotyl length (**d**) in a dose dependent manner and requires an activated and functional $SCF^{TIR1/AFB}$ complex (means ± s.e. are shown, n = 20). (**e**) Representative images of the *hookback* mutants in presence of DR4. (**f**) Maximal intensity projection of a 48-hour time course of the *hookback* mutants in control condition. (**g**) Side and top view of four-week-old *hookback* mutants grown in soil. Concentrations are indicated in µM between brackets.

3.3 Digital image processing to study cellulose biosynthesis and pavement cell shape

3.3.1 Density estimation and colocalization (Paper II)

Plants use regulated cell expansion during development and to adapt their form to environmental conditions. The cell wall is composed of long, load-bearing cellulose microfibrils embedded in a viscoelastic matrix of pectins, hemicelluloses, and structural proteins (Baskin, 2005; Lerouxel *et al.*, 2006). Cellulose consists of a paracrystalline assembly of parallel β-1,4-linked glucan chains, which coalesce to form microfibrils (Guerriero *et al.*, 2010). In land plants, cellulose is synthesized from an hexameric membrane-bound protein complex named Cellulose Synthase Complex (CSC) (Guerriero *et al.*, 2010). The Arabidopsis genome contains at least 10 different *CELLULOSE SYNTHASE* (*CESA*) genes (Richmond, 2000). Another protein essential for cellulose biosynthesis is a membrane-bound endo-1,4- β -D-glucanase, called KORRIGAN1 (KOR1) (Nicol *et al.*, 1998). Genetic studies indicate that KOR1 is required for cellulose synthesis in primary and secondary cell walls (Nicol *et al.*, 1998; Lane *et al.*, 2001; Peng *et al.*, 2001; Szyjanowicz *et al.*, 2004). GFP-KOR1 in living cells was found in discrete particles at the plasma membrane as it was described for GFP-CESA (Paredez *et al.*, 2006; Crowell *et al.*, 2009; Gutierrez *et al.*, 2009). KOR1 is also present in different intracellular compartments (Robert *et al.*, 2005). These findings are similar to those observed in CESAs suggesting that KOR1 might be part of the CSC.

GFP-CESA3 proteins are present as fluorescent punctuate at the plasma membrane and move bidirectionally at a constant average of ~250 $nm.min^{-1}$ (Paredez *et al.*, 2006; Pochylova *et al.*, 2007; Bischoff *et al.*, 2009; Crowell *et al.*, 2009). The impact of KOR1 on GFP-CESA3 velocity was investigated by expressing GFP-CESA3 in the *kor1-1* mutants. Interestingly, the velocity of the GFP-CESA3 was significantly reduced in the absence of a functional KOR1. The spinning disk microscopy approach has been used to study the

dynamics of the GFP-CESA3 proteins. This kind of microscopy has great advantages in monitoring fluorescent proteins dynamics and reduces photobleaching. However, the resulted images are very noisy. The GFP-CESA3 signal, when recorded at the plasma membrane comprised at least the small particles described as CSC and the GFP-CESA3 labelled Golgi apparatus. Thus, digital image processing has been developed to (i) restore image, (ii) select area without Golgi signal and (iii) extract and measure the surface covered by the GFP-CESA3 at the plasma membrane. Thus, we showed that KOR1 did not influence the surface covered by the GFP-CESA3 proteins at the plasma membrane. However, internalization of GFP-CESA3 in microtubule-associated compartment (MASC) upon treatment with a cellulose synthesis inhibitor was reduced in *kor1-1* mutant background demonstrating a role of KOR1 for the intracellular trafficking of the CSCs. In order to determine if CSCs could interact with KOR1, we analysed the colocalization of mCherry-CESA1 and GFP-KOR1 at the plasma membrane. Dual-channel images were denoised and processed using the TopHat transformation. Subsequently, we computed the Pearson's coefficient to estimate the correlation between the two processed images. As control, we rotated one channel to 90 degrees, which resulted in a coefficient close to 0 corresponding to a random correlation. Thus, we concluded that KOR1 and CSC partially colocalised at the plasma membrane. Split Ubiquitin Yeast Two Hybrid and Bimolecular Fluorescence complementation confirmed the direct interaction of KOR1 with the CSC at the plasma membrane.

3.3.2 Pavement cell shape analysis (Paper III)

Arabidopsis leaf pavement cells have jigsaw puzzle geometry. The formation of interdigitating lobes between adjacent cells requires localised outgrowth and cell wall extension (Fu *et al.*, 2002, 2005). Pavement cell shapes vary in term of surface and their geometrical complexity increases with their size (Armour *et al.*, 2015). A semi-automated image analysis workflow has been developed to measure the geometrical features of a pavement cell population. To label the cell outline, propidium iodide, a cell wall dye, was applied on fixed samples. First, and to facilitate the automated image segmentation, a pre-processing of the pictures was performed to remove the background and increase the contrast of the cell outlines. The semi-automated segmentation was conducted using watershed with the CellSet software (Pound *et al.*, 2012), as it allows manual corrections. The output of such segmentation was a binary image of the cell outlines. The stomata were removed (coloured as background) to consider only pavement cells in the analysis. Then, the surface and circularity of each pavement cell were measured to characterise the shape of the pavement cell

population. The skeletonization of the cell shape was performed and the edge number of the skeleton was considered as an estimator of the lobe number. Such factor has recently been used to monitor pavement cell growth (Armour *et al.*, 2015). The skeletonization has been described to underestimate the lobe number especially for large pavement cells, which are highly interdigitated (Wu *et al.*, 2016). The rapid computation of a large number of cells (1928 > n > 350) without a potential bias introduced by manual measurements provided a robust quantitative strategy to analyse pavement cell geometry. By this semi-automated approach, sixteen cell wall deficient mutants have been analysed systematically and these data demonstrated the interdependence of cell wall component synthesis and/or remodelling to control the growth and the interdigitation level of pavement cells.

Conclusions and Future Perspectives

In **paper I**, by forward chemical screen we isolated 34 compounds acting through the AXR1-mediated signalling pathway. Among them, we characterized a new type of auxin related molecules acting as selective agonists. This work confirmed the potential to modulate various developmental features by affecting Aux/IAA turnover. DR compounds target only some specific paths within the complex signalling network transducing auxin perception. Thus, the choice of one DR associated to its specific effect should lead to isolate functional components of the auxin signalling pathway. Detailed reverse genetic screen using mutated Aux/IAA and ARFs proteins could be performed to determine their involvement in the developmental defects induced by the DRs. Moreover, the isolation of DR-resistant mutants will allow the isolation of novel molecular players in specific developmental processes. In this work, we show the potential of DR4 to act through genes controlling developmentally programed differential growth. The characterization of mutations conferring resistance to DR4 will certainly lead to previously inaccessible auxin-related molecular regulation.

Induction of phenotypes by applying a chemical compound is a powerful tool for studying plant development. However, the chemical nature of the agent used lead to complex questions regarding chemical stability, uptake, metabolism and toxicity. Characterizing the chemical permeability and stability within living organism is a challenging question that we addressed in **paper I**. The analysis of the DR-degradation products and DR-analogues in term of effect and target affinity demonstrated the importance of each functional group for the DR-effects. In the case of this study, such analysis confirmed that pro-drug properties of DR compounds were not sufficient to modulate the observed effect on seedling development. The DR structures have the potential to serve as a base to generate biased chemical libraries and extend the prospection of the chemical space related to auxin biology.

The digital image processing used in **paper II** and **paper III** exemplifies the basic workflow required to extract quantitative data from digital images. The developments of automated microscopes and computer controlled cameras allow the possibility to perform phenotyping from a tremendous amount of diverse images.

References

Adamowski, M. & Friml, J., 2015. PIN-dependent auxin transport: action, regulation, and evolution. *The Plant cell*, 27(1), pp.20–32.

Ahn, Y.-H., Lee, J.-S. & Chang, Y.-T., 2007. Combinatorial Rosamine Library and Application to in Vivo Glutathione Probe. *Journal of the American Chemical Society*, 129(15), pp.4510–4511.

Alonso-Blanco, C. et al., 2016. 1,135 Genomes Reveal the Global Pattern of Polymorphism in Arabidopsis thaliana. *Cell*, 166(2), pp.481–491.

Armengot, L., Marquès-Bueno, M.M. & Jaillais, Y., 2016. Regulation of polar auxin transport by protein and lipid kinases. *Journal of experimental botany*, 67(14), pp.4015–37.

Armour, W.J. et al., 2015. Differential Growth in Periclinal and Anticlinal Walls during Lobe Formation in Arabidopsis Cotyledon Pavement Cells. *The Plant cell*, 27(9), pp.2484–500.

Armstrong, J.I. et al., 2004. Identification of inhibitors of auxin transcriptional activation by means of chemical genetics in Arabidopsis. *Proceedings of the National Academy of Sciences of the United States of America*, 101(41), pp.14978–83.

Austin, C.P. et al., 2004. NIH Molecular Libraries Initiative. *Science*, 306(5699), pp.1138–9.

Bachovchin, D.A. et al., 2014. A high-throughput, multiplexed assay for superfamily-wide profiling of enzyme activity. *Nature Chemical Biology*, 10(8), pp.656–663.

Bachovchin, D.A. et al., 2009. Identification of selective inhibitors of uncharacterized enzymes by high-throughput screening with fluorescent activity-based probes. *Nature biotechnology*, 27(4), pp.387–394.

Backman, T.W.H., Cao, Y. & Girke, T., 2011. ChemMine tools: an online service for analyzing and clustering small molecules. *Nucleic acids research*, 39(Web Server issue), pp.W486-91.

Balkenhohl, F. et al., 1996. Combinatorial Synthesis of Small Organic Molecules. *Angewandte Chemie International Edition in English*, 35(20), pp.2288–2337.

Barbez, E. et al., 2012. A novel putative auxin carrier family regulates intracellular auxin homeostasis in plants. *Nature*, 485(7396), pp.119–22.

Baskin, T.I., 2005. Anisotropic Expansion of the Plant Cell Wall. *Annual Review of Cell and Developmental Biology*, 21(1), pp.203–222.

Bennett, M.J. et al., 1996. Arabidopsis AUX1 gene: a permease-like regulator of root gravitropism. *Science (New York, N.Y.)*, 273(5277), pp.948–50.

Bischoff, V. et al., 2009. Thaxtomin A affects CESA-complex density, expression of cell wall genes, cell wall composition, and causes ectopic lignification in Arabidopsis thaliana seedlings. *Journal of experimental botany*, 60(3), pp.955–65.

Boer, D.R. et al., 2014. Structural Basis for DNA Binding Specificity by the Auxin-Dependent ARF Transcription Factors. *Cell*, 156(3), pp.577–589.

Bohacek, R.S., McMartin, C. & Guida, W.C., 1996. The art and practice of structure-based drug design: A molecular modeling perspective. *Medicinal Research Reviews*, 16(1), pp.3–50.

Bolte, S. & Cordelières, F.P., 2006. A guided tour into subcellular colocalization analysis in light microscopy. *Journal of microscopy*, 224(December), pp.213–232.

Borgstrom, G., 1939. Influence of growth-promoting chemicals on roots of allium. I. Effective concentration ranges of synthetic growth substances and vitamins. *Botaniska Notiser*, pp.207–220.

Boulanger, J., Kervrann, C. & Bouthemy, P., 2007. Space-Time Adaptation for Patch-Based Image Sequence Restoration. *IEEE Transactions on Pattern Analysis and Machine Intelligence*, 29(6), pp.1096–1102.

Calderón Villalobos, L.I.A. et al., 2012. A combinatorial TIR1/AFB-Aux/IAA co-receptor system for differential sensing of auxin. *Nature chemical biology*, 8(5), pp.477–85.

Calderon-Villalobos, L.I. et al., 2010. Auxin perception--structural insights. *Cold Spring Harbor perspectives in biology*, 2(7), p.a005546.

Charleston, M.A. & Robertson, D.L., 2002. Preferential host switching by primate lentiviruses can account for phylogenetic similarity with the primate phylogeny. *Systematic biology*, 51(3), pp.528–535.

Chilton, M.D. et al., 1977. Stable incorporation of plasmid DNA into higher plant cells: the molecular basis of crown gall tumorigenesis. *Cell*, 11(2), pp.263–71.

Christian, M. et al., 2008. Identification of auxins by a chemical genomics approach. *Journal of experimental botany*, 59(10), pp.2757–67.

Crowell, E.F. et al., 2009. Pausing of Golgi Bodies on Microtubules Regulates Secretion of Cellulose Synthase Complexes in Arabidopsis. *The Plant cell*, 21(4), pp.1–14.

Darwin, C. & Darwin, F., 1881. *The power of movement in plants.*, New York,: Appleton,.

Dayan, F.E., Cantrell, C.L. & Duke, S.O., 2009. Natural products in crop protection. *Bioorganic & Medicinal Chemistry*, 17(12), pp.4022–4034.

Dejonghe, W. & Russinova, E., 2014. Target identification strategies in plant chemical biology. *Frontiers in plant science*, 5, p.352.

Dharmasiri, N., Dharmasiri, S. & Estelle, M., 2005. The F-box protein TIR1 is an auxin receptor. *Nature*, 435(7041), pp.441–5.

Dobson, C.M., 2004. Chemical space and biology. *Nature*, 432(7019), pp.824–828.

Downes, B. & Vierstra, R.D., 2005. Post-translational regulation in plants employing a diverse set of polypeptide tags. *Biochemical Society transactions*, 33(Pt 2), pp.393–9.

Doyle, S.M., Vain, T. & Robert, S., 2015. Small molecules unravel complex interplay between auxin biology and endomembrane trafficking. *Journal of Experimental Botany*, 66(16), pp.4971–4982.

Drakakaki, G. & Robert, S., 2011. Clusters of bioactive compounds target dynamic endomembrane networks in vivo. *Proceedings of the National Academy of Sciences of the United States of America*, 108 (43) pp. 17850-17855.

Dreher, K.A. et al., 2006. The Arabidopsis Aux/IAA protein family has diversified in degradation and auxin responsiveness. *The Plant cell*, 18(3), pp.699–714.

Enchev, R.I., Schulman, B.A. & Peter, M., 2015. Protein neddylation: beyond cullin-RING ligases. *Nature reviews. Molecular cell biology*, 16(1), pp.30–44.

Enders, T.A. & Strader, L.C., 2015. Auxin activity: Past, present, and future. *American journal of botany*, 102(2), pp.180–96.

Estelle, M.A. & Somerville, C., 1987. Auxin-resistant mutants of Arabidopsis thaliana with an altered morphology. *MGG Molecular & General Genetics*, 206(2), pp.200–206.

Estelle, M.A. & Somerville, C.R., 1986. The mutants of Arabidopsis. *Trends in Genetics*, 2, pp.89–93.

Eyer, L. et al., 2016. 2,4-D and IAA Amino Acid Conjugates Show Distinct Metabolism in Arabidopsis. *PloS one*, 11(7), p.e0159269.

Friml, J. et al., 2002a. AtPIN4 mediates sink-driven auxin gradients and root patterning in Arabidopsis. *Cell*, 108(5), pp.661–73.

Friml, J. et al., 2002b. Lateral relocation of auxin efflux regulator PIN3 mediates tropism in Arabidopsis. *Nature*, 415(6873), pp.806–809.

Friml, J., 2003. Auxin transport — shaping the plant. *Current Opinion in Plant Biology*, 6(1), pp.7–12.

Fu, Y. et al., 2005. Arabidopsis interdigitating cell growth requires two antagonistic pathways with opposing action on cell morphogenesis. *Cell*, 120(5), pp.687–700.

Fu, Y., Li, H. & Yang, Z., 2002. The ROP2 GTPase controls the formation of cortical fine F-actin and the early phase of directional cell expansion during Arabidopsis organogenesis. *The Plant cell*, 14(4), pp.777–94.

Gan, X. et al., 2011. Multiple reference genomes and transcriptomes for Arabidopsis thaliana. *Nature*, 477(7365), pp.419–423.

Geisler, M. et al., 2005. Cellular efflux of auxin catalyzed by the Arabidopsis MDR/PGP transporter AtPGP1. *The Plant journal : for cell and molecular biology*, 44(2), pp.179–94.

Geisler, M. et al., 2003. TWISTED DWARF1, a unique plasma membrane-anchored immunophilin-like protein, interacts with Arabidopsis multidrug resistance-like transporters AtPGP1 and AtPGP19. *Molecular biology of the cell*, 14(10), pp.4238–49.

Gilkerson, J. et al., 2009. Isolation and characterization of cul1-7, a recessive allele of CULLIN1 that disrupts SCF function at the C terminus of CUL1 in Arabidopsis thaliana. *Genetics*, 181(3), pp.945–63.

Gillet, V.J., 2008. New directions in library design and analysis. *Current Opinion in Chemical Biology*, 12(3), pp.372–378.

Grosdidier, A., Zoete, V. & Michielin, O., 2011. SwissDock, a protein-small molecule docking web service based on EADock DSS. *Nucleic acids research*, 39(Web Server issue), pp.W270-7.

Guerriero, G., Fugelstad, J. & Bulone, V., 2010. Invited Expert Review What Do We Really Know about Cellulose Biosynthesis in Higher Plants ? *Journal of integrative plant biology*, 52(2), pp.161–175.

Gutierrez, R. et al., 2009. Arabidopsis cortical microtubules position cellulose synthase delivery to the plasma membrane and interact with cellulose synthase trafficking compartments. *Nature cell biology*, 11(June), pp.797–806.

Gälweiler, L. et al., 1998. Regulation of polar auxin transport by AtPIN1 in Arabidopsis vascular tissue. *Science (New York, N.Y.)*, 282(5397), pp.2226–30.

Hall, M.D. et al., 2016. Fluorescence polarization assays in high-throughput screening and drug discovery: a review. *Methods and Applications in Fluorescence*, 4(2), p.22001.

Havens, K.A. et al., 2012. A synthetic approach reveals extensive tunability of auxin signaling. *Plant physiology*, 160(1), pp.135–42.

Hayashi, K. et al., 2012. Rational design of an auxin antagonist of the SCF(TIR1) auxin receptor complex. *ACS chemical biology*, 7(3), pp.590–8.

Hobbie, L. et al., 2000. The axr6 mutants of Arabidopsis thaliana define a gene involved in auxin response and early development. *Development (Cambridge, England)*, 127(1), pp.23–32.

Hobbie, L. & Estelle, M., 1995. The axr4 auxin-resistant mutants of Arabidopsis thaliana define a gene important for root gravitropism and lateral root initiation. *The Plant journal : for cell and molecular biology*, 7(2), pp.211–20.

Hochstrasser, M., 2009. Origin and function of ubiquitin-like proteins. *Nature*, 458(7237), pp.422–9.

Hotton, S.K. et al., 2011. AXR1-ECR1 and AXL1-ECR1 heterodimeric RUB-activating enzymes diverge in function in Arabidopsis thaliana. *Plant molecular biology*, 75(4–5), pp.515–26.

Hotton, S.K. & Callis, J., 2008. Regulation of cullin RING ligases. *Annual review of plant biology*, 59, pp.467–89.

Hu, Z. et al., 2012. F-box protein AFB4 plays a crucial role in plant growth, development and innate immunity. *Cell research*, 22(4), pp.777–81.

Hua, Z. & Vierstra, R.D., 2011. The cullin-RING ubiquitin-protein ligases. *Annual review of plant biology*, 62, pp.299–334.

Irwin, J.J. et al., 2012. ZINC: A Free Tool to Discover Chemistry for Biology. *Journal of Chemical Information and Modeling*, 52(7), pp.1757–1768.

Jacob, C. & Walters, A., 2005. Risk and Responsibility in Chemical Research: The Case of Agent Orange. *HYLE – International Journal for Philosophy of Chemistry*, 11(2), pp.147–166.

Jeschke, P., 2016. Progress of modern agricultural chemistry and future prospects. *Pest Management Science*, 72(3), pp.433–455.

Jones, A.M. et al., 2008. The Impact of Arabidopsis on Human Health: Diversifying Our Portfolio. *Cell*, 133(6), pp.939–943.

Kanoh, N., 2016. Photo-cross-linked small-molecule affinity matrix as a tool for target identification of bioactive small molecules. *Natural product reports*, 33(5), pp.709–18.

Kasahara, H., 2015. Current aspects of auxin biosynthesis in plants. *Bioscience, Biotechnology, and Biochemistry*, pp.1–9.

Kelley, D.R. & Estelle, M., 2012. Ubiquitin-mediated control of plant hormone signaling. *Plant physiology*, 160(1), pp.47–55.

Kepinski, S., 2009. Pull-down assays for plant hormone research. *Methods in molecular biology (Clifton, N.J.)*, 495, pp.61–80.

Kepinski, S. & Leyser, O., 2005. The Arabidopsis F-box protein TIR1 is an auxin receptor. *Nature*, 435(7041), pp.446–51.

Kerscher, O., Felberbaum, R. & Hochstrasser, M., 2006. Modification of proteins by ubiquitin and ubiquitin-like proteins. *Annual review of cell and developmental biology*, 22, pp.159–80.

Kim, J.-Y. et al., 2010. Identification of an ABCB/P-glycoprotein-specific inhibitor of auxin transport by chemical genomics. *The Journal of biological chemistry*, 285(30), pp.23309–17.

Kim, S. et al., 2015. PubChem structure-activity relationship (SAR) clusters. *Journal of cheminformatics*, 7, p.33.

Kirsch, R.A. et al., 1958. Experiments in processing pictorial information with a digital computer. In *Papers and discussions presented at the December 9-13, 1957, eastern joint computer conference: Computers with deadlines to meet on XX - IRE-ACM-AIEE '57 (Eastern)*. New York, New York, USA: ACM Press, pp. 221–229.

Klíma, P., Laňková, M. & Zažímalová, E., 2015. Inhibitors of plant hormone transport. *Protoplasma*, pp.1–14.

Krogan, N.T., Hogan, K. & Long, J.A., 2012. APETALA2 negatively regulates multiple floral organ identity genes in Arabidopsis by recruiting the co-repressor TOPLESS and the histone deacetylase HDA19. *Development*, 139(22).

Krouk, G. et al., 2010. Nitrate-Regulated Auxin Transport by NRT1.1 Defines a Mechanism for Nutrient Sensing in Plants. *Developmental Cell*, 18(6), pp.927–937.

Kögl, F., Haagen-Smit, A.J. & Erxleben, H., 1934. Über ein neues Auxin („Hetero-auxin") aus Harn. 11. Mitteilung über pflanzliche Wachstumsstoffe. *Hoppe-Seyler´s Zeitschrift für physiologische Chemie*, 228(1–2), pp.90–103.

Laibach, F., 1943. Arabidopsis thaliana (L.) Heynh. als object fur genetische und entwicklungsphysiologische untersuchungen. *Bot. Archiv.*, 44, pp.439–455.

Laibach, F., 1907. Zur frage nach der individualität der chromosomen im plfanzenreich. *Beih. Bot. Zentralbl.*, 22, pp.191–210.

Lamberth, C. et al., 2013. Current challenges and trends in the discovery of agrochemicals. *Science (New York, N.Y.)*, 341(6147), pp.742–6.

Lane, D.R. et al., 2001. Temperature-sensitive alleles of RSW2 link the KORRIGAN endo-1,4-beta-glucanase to cellulose synthesis and cytokinesis in Arabidopsis. *Plant physiology*, 126(1), pp.278–88.

Larebeke, N. et al., 1975. Acquisition of tumour-inducing ability by non-oncogenic agrobacteria as a result of plasmid transfer. *Nature*, 255(5511), pp.742–743.

Lavy, M. et al., 2016. Constitutive auxin response in Physcomitrella reveals complex interactions between Aux/IAA and ARF proteins. *eLife*, 5.

Lee, J. & Bogyo, M., 2013. Target deconvolution techniques in modern phenotypic profiling. *Current Opinion in Chemical Biology*, 17(1), pp.118–126.

Lee, S. et al., 2014. Defining binding efficiency and specificity of auxins for SCF(TIR1/AFB)-Aux/IAA co-receptor complex formation. *ACS chemical biology*, 9(3), pp.673–82.

Léran, S. et al., 2014. A unified nomenclature of NITRATE TRANSPORTER 1/PEPTIDE TRANSPORTER family members in plants. *Trends in Plant Science*, 19(1), pp.5–9.

Lerouxel, O. et al., 2006. Biosynthesis of plant cell wall polysaccharides — a complex process. *Current Opinion in Plant Biology*, 9(6), pp.621–630.

Levan, A., 1939. Cytological phenomena connected with the root swelling caused by growth substances. *Hereditas*, 25(1), pp.87–96.

Leyser, H.M. et al., 1993. Arabidopsis auxin-resistance gene AXR1 encodes a protein related to ubiquitin-activating enzyme E1. *Nature*, 364(6433), pp.161–4.

Leyser, H.M.O. et al., 1996. Mutations in the AXR3 gene of Arabidopsis result in altered auxin response including ectopic expression from the SAUR-AC1 promoter. *The Plant Journal*, 10(3), pp.403–413.

Lincoln, C., Britton, J.H. & Estelle, M., 1990. Growth and development of the axr1 mutants of Arabidopsis. *The Plant cell*, 2(11), pp.1071–80.

Lipinski, C. & Hopkins, A., 2004. Navigating chemical space for biology and medicine. *Nature*, 432(7019), pp.855–861.

Ljung, K., 2013. Auxin metabolism and homeostasis during plant development. *Development*, 140(5).

Lloyd, A.M. et al., 1986. Transformation of Arabidopsis thaliana with Agrobacterium tumefaciens. *Science (New York, N.Y.)*, 234(4775), pp.464–6.

Lowrie, J. et al., 2004. The Different Strategies for Designing GPCR and Kinase Targeted Libraries. *Combinatorial Chemistry & High Throughput Screening*, 7(5), pp.495–510.

Luschnig, C. & Vert, G., 2014. The dynamics of plant plasma membrane proteins: PINs and beyond. *Development (Cambridge, England)*, 141(15), pp.2924–38.

Ma, Q. & Robert, S., 2014. Auxin biology revealed by small molecules. *Physiologia Plantarum*, 151(1), pp.25–42.

Maher, E.P. & Martindale, S.J.B., 1980. Mutants of Arabidopsis thaliana with altered responses to auxins and gravity. *Biochemical genetics*, 18(11–12), pp.1041–53.

Manders, E.M.M., Verbeek, F.J. & Aten, J.A., 1993. Measurement of co-localization of objects in dual-colour confocal images. *Journal of Microscopy*, 169(3), pp.375–382.

Maraschin, F. dos S., Memelink, J. & Offringa, R., 2009. Auxin-induced, SCF(TIR1)-mediated poly-ubiquitination marks AUX/IAA proteins for degradation. *The Plant journal : for cell and molecular biology*, 59(1), pp.100–9.

Marchant, A. et al., 1999. AUX1 regulates root gravitropism in Arabidopsis by facilitating auxin uptake within root apical tissues. *The EMBO journal* , 18(8), pp.2066–2073.

Matsen, F.A. et al., 2015. Tanglegrams: a reduction tool for mathematical phylogenetics.

Mayr, L.M. & Bojanic, D., 2009. Novel trends in high-throughput screening. *Current Opinion in Pharmacology*, 9(5), pp.580–588.

McCourt, P. & Desveaux, D., 2010. Plant chemical genetics. *New Phytologist*, 185(1), pp.15–26.

Mellor, N. et al., 2016. Dynamic regulation of auxin oxidase and conjugating enzymes AtDAO1 and GH3 modulates auxin homeostasis. *Proceedings of the National Academy of Sciences of the United States of America*, p.201604458.

Mergner, J. & Schwechheimer, C., 2014. The NEDD8 modification pathway in plants. *Frontiers in plant science*, 5, p.103. rg/article/10.3389/fpls.2014.00103/abstract [Accessed July 21, 2015].

Meyerowitz, E.M., 1987. Arabidopsis thaliana. *Annual review of genetics*, 21, pp.93–111.

Meyerowitz, E.M. & Pruitt, R.E., 1985. Arabidopsis thaliana and Plant Molecular Genetics. *Science (New York, N.Y.)*, 229(4719), pp.1214–8.

Mironova, V. V et al., 2014. Computational analysis of auxin responsive elements in the Arabidopsis thaliana L. genome. *BMC genomics*, (Suppl 12), p.S4.

Mishev, K., Dejonghe, W. & Russinova, E., 2013. Small Molecules for Dissecting Endomembrane Trafficking: A Cross-Systems View. *Chemistry & Biology*, 20(4), pp.475–486.

Mitchison, T.J., 1994. Towards a pharmacological genetics. *Chemistry & Biology*, 1(1), pp.3–6.

Mithila, J. et al., 2011. Evolution of Resistance to Auxinic Herbicides: Historical Perspectives, Mechanisms of Resistance, and Implications for Broadleaf Weed Management in Agronomic Crops. *Weed Science*, 59(4), pp.445–457.

Moon, J. et al., 2007. A new CULLIN 1 mutant has altered responses to hormones and light in Arabidopsis. *Plant physiology*, 143(2), pp.684–96.

Mosquna, A. et al., 2011. Potent and selective activation of abscisic acid receptors in vivo by mutational stabilization of their agonist-bound conformation. *Proceedings of the National Academy of Sciences of the United States of America*, 108(51), pp.20838–43.

Moss, B.L. et al., 2015. Rate motifs tune Aux/IAA degradation dynamics. *Plant physiology*. pp.00587.2015

Müller, A. et al., 1998. AtPIN2 defines a locus of Arabidopsis for root gravitropism control. *The EMBO journal*, 17(23), pp.6903–11.

Nicol, F. et al., 1998. A plasma membrane-bound putative endo-1 , 4- β - D - glucanase is required for normal wall assembly and cell elongation in Arabidopsis. *The EMBO journal*, 17(19), pp.5563–5576.

Niphakis, M.J. & Cravatt, B.F., 2014. Enzyme Inhibitor Discovery by Activity-Based Protein Profiling. *Annual Review of Biochemistry*, 83(1), pp.341–377.

Noh, B., Murphy, A.S. & Spalding, E.P., 2001. Multidrug resistance-like genes of Arabidopsis required for auxin transport and auxin-mediated development. *The Plant cell*, 13(11), pp.2441–54.

Paponov, I.A. et al., 2009. The evolution of nuclear auxin signalling. *BMC Evolutionary Biology*, 9(1), p.126.

Paredez, A.R., Somerville, C.R. & Ehrhardt, D.W., 2006. Visualization of cellulose synthase demonstrates functional association with microtubules. *Science (New York, N.Y.)*, 312(5779), pp.1491–5.

Park, S.-Y. et al., 2009. Abscisic acid inhibits type 2C protein phosphatases via the PYR/PYL family of START proteins. *Science (New York, N.Y.)*, 324(5930), pp.1068–71.

Park, S.-Y. et al., 2015. Agrochemical control of plant water use using engineered abscisic acid receptors. *Nature*, 520(7548), pp.545–548.

Parry, G. et al., 2009. Complex regulation of the TIR1/AFB family of auxin receptors. *Proceedings of the National Academy of Sciences of the United States of America*, 106(52), pp.22540–5.

Pencík, A. et al., 2013. Regulation of auxin homeostasis and gradients in Arabidopsis roots through the formation of the indole-3-acetic acid catabolite 2-oxindole-3-acetic acid. *The Plant cell*, 25(10), pp.3858–70.

Peng, L. et al., 2001. The Experimental Herbicide CGA 325 , 615 Inhibits Synthesis of Crystalline Cellulose and Causes Accumulation of Non-Crystalline ␣ -1 , 4-Glucan Associated with CesA Protein 1. , 126(July), pp.981–992.

Péret, B. et al., 2012. AUX/LAX genes encode a family of auxin influx transporters that perform distinct functions during Arabidopsis development. *The Plant cell*, 24(7), pp.2874–85.

Petrásek, J. & Friml, J., 2009. Auxin transport routes in plant development. *Development (Cambridge, England)*, 136(16), pp.2675–88.

Petroski, M.D. & Deshaies, R.J., 2005. Function and regulation of cullin-RING ubiquitin ligases. *Nature reviews. Molecular cell biology*, 6(1), pp.9–20.

Pickart, C.M. & Cohen, R.E., 2004. Proteasomes and their kin: proteases in the machine age. *Nature reviews. Molecular cell biology*, 5(3), pp.177–87.

Pickett, F.B., Wilson, A.K. & Estelle, M., 1990. The aux1 Mutation of Arabidopsis Confers Both Auxin and Ethylene Resistance. *Plant physiology*, 94(3), pp.1462–6.

Piya, S. et al., 2014. Protein-protein interaction and gene co-expression maps of ARFs and Aux/IAAs in Arabidopsis. *Frontiers in plant science*, 5, p.744.

Pochylova, Z. et al., 2007. Organization of cellulose synthase complexes involved in primary cell wall synthesis in Arabidopsis thaliana. *Proceedings of the National Academy of Sciences of the United States of America*, 104(39), pp.1–6.

Pokorny, R., 1941. New Compounds. Some Chlorophenoxyacetic Acids. *Journal of the American Chemical Society*, 63(6), pp.1768–1768.

Porco, S. et al., 2016. Dioxygenase-encoding AtDAO1 gene controls IAA oxidation and homeostasis in Arabidopsis. *Proceedings of the National Academy of Sciences of the United States of America*, p.201604375.

Pound, M.P. et al., 2012. CellSeT: novel software to extract and analyze structured networks of plant cells from confocal images. *The Plant cell*, 24(4), pp.1353–61.

Pozo, J.C. et al., 1998. The ubiquitin-related protein RUB1 and auxin response in Arabidopsis. *Science (New York, N.Y.)*, 280(5370), pp.1760–3.

del Pozo, J.C. & Estelle, M., 1999. The Arabidopsis cullin AtCUL1 is modified by the ubiquitin-related protein RUB1. *Proceedings of the National Academy of Sciences of the United States of America*, 96(26), pp.15342–7.

Del Pozo, J.C. & Manzano, C., 2014. Auxin and the ubiquitin pathway. Two players-one target: the cell cycle in action. *Journal of experimental botany*, 65(10), pp.2617–32.

Prigge, M.J. et al., 2016. The Arabidopsis Auxin Receptor F-Box Proteins AFB4 and AFB5 Are Required for Response to the Synthetic Auxin Picloram. *G3: Genes|Genomes|Genetics*, 6(5), p.1383.

Provart, N.J. et al., 2016. 50 years of Arabidopsis research: highlights and future directions. *New Phytologist*, 209(3), pp.921–944.

Rampey, R.A. et al., 2013. Compensatory mutations in predicted metal transporters modulate auxin conjugate responsiveness in Arabidopsis. *G3 (Bethesda, Md.)*, 3(1), pp.131–41.

Rautio, J. et al., 2008. Prodrugs: design and clinical applications. *Nature Reviews Drug Discovery*, 7(3), pp.255–270.

Raven, J.A., 1975. Transport of indoleacetic acid in plant cells in relation to ph and electrical potential gradients, and its significance for polar iaa transport. *New Phytologist*, 74(2), pp.163–172.

Reed, J.W., 2001. Roles and activities of Aux/IAA proteins in Arabidopsis. *Trends in Plant Science*, 6(9), pp.420–425.

Richmond, T., 2000. Higher plant cellulose synthases. *Genome biology*, 1(4), p.REVIEWS3001.

Robert, S. et al., 2005. An Arabidopsis endo-1,4-beta-D-glucanase involved in cellulose synthesis undergoes regulated intracellular cycling. *The Plant cell*, 17(12), pp.3378–89.

Robert, S. et al., 2008. Endosidin1 defines a compartment involved in endocytosis of the brassinosteroid receptor BRI1 and the auxin transporters PIN2 and AUX1. *Proceedings of the National Academy of Sciences*, 105(24), pp.8464–8469.

Robert, S., Raikhel, N. V & Hicks, G.R., 2009. Powerful partners: Arabidopsis and chemical genomics. *The Arabidopsis book / American Society of Plant Biologists*, 7, p.e0109.

Rubery, P.H. & Sheldrake, A.R., 1974. Carrier-mediated auxin transport. *Planta*, 118(2), pp.101–21.

Ruegger, M. et al., 1997. Reduced naphthylphthalamic acid binding in the tir3 mutant of Arabidopsis is associated with a reduction in polar auxin transport and diverse morphological defects. *The Plant Cell*, 9(5), p.745.

Ruegger, M. et al., 1998. The TIR1 protein of Arabidopsis functions in auxin response and is related to human SKP2 and yeast grr1p. *Genes & development*, 12(2), pp.198–207.

Ruegger, M. et al., 1998. The TIR1 protein of Arabidopsis functions in auxin response and is related to human SKP2 and yeast grr1p. *Genes & development*, 12(2), pp.198–207.

De Rybel, B. et al., 2012. A role for the root cap in root branching revealed by the non-auxin probe naxillin. *Nature chemical biology*, 8(9), pp.798–805.

De Rybel, B. et al., 2009. Chemical Inhibition of a Subset of Arabidopsis thaliana GSK3-like Kinases Activates Brassinosteroid Signaling. *Chemistry & Biology*, 16(6), pp.594–604.

Sachs, J., 1890. *History of botany (1530-1860)*, Oxford,: Clarendon Press,.

Salehin, M., Bagchi, R. & Estelle, M., 2015. SCFTIR1/AFB-based auxin perception: mechanism and role in plant growth and development. *The Plant cell*, 27(1), pp.9–19.

Santner, A. & Estelle, M., 2010. The ubiquitin-proteasome system regulates plant hormone signaling. *The Plant journal : for cell and molecular biology*, 61(6), pp.1029–40.

Sarikas, A., Hartmann, T. & Pan, Z.-Q., 2011. The cullin protein family. *Genome biology*, 12(4), p.220.

Sauer, M. et al., 2006. Canalization of auxin flow by Aux/IAA-ARF-dependent feedback regulation of PIN polarity. *Genes & development*, 20(20), pp.2902–11.

Savaldi-Goldstein, S. et al., 2008. New auxin analogs with growth-promoting effects in intact plants reveal a chemical strategy to improve hormone delivery. *Proceedings of the National Academy of Sciences of the United States of America*, 105(39), pp.15190–5.

Schenone, M. et al., 2013. Target identification and mechanism of action in chemical biology and drug discovery. *Nature chemical biology*, 9(4), pp.232–40.

Schrader, J. et al., 2003. Polar auxin transport in the wood-forming tissues of hybrid aspen is under simultaneous control of developmental and environmental signals. *Proceedings of the National Academy of Sciences of the United States of America*, 100(17), pp.10096–101.

Schreiber, S.L. et al., 2015. Advancing Biological Understanding and Therapeutics Discovery with Small-Molecule Probes. *Cell*, 161(6), pp.1252–1265.

Schwechheimer, C. et al., 2001. Interactions of the COP9 signalosome with the E3 ubiquitin ligase SCFTIRI in mediating auxin response. *Science (New York, N.Y.)*, 292(5520), pp.1379–82.

Scornavacca, C., Zickmann, F. & Huson, D.H., 2011. Tanglegrams for rooted phylogenetic trees and networks. *Bioinformatics (Oxford, England)*, 27(13), pp.i248-56.

Serra, J.P., 1982. Image analysis and mathematical morphology, Academic Press.

Simeonov, A. et al., 2008. Fluorescence Spectroscopic Profiling of Compound Libraries. *Journal of Medicinal Chemistry*, 51(8), pp.2363–2371.

Simon, S. et al., 2013. Defining the selectivity of processes along the auxin response chain: a study using auxin analogues. *New Phytologist*, 200(4), pp.1034–1048.

Simon, S. & Petrášek, J., 2011. Why plants need more than one type of auxin. *Plant Science*, 180(3), pp.454–460.

Skaar, J.R. & Pagano, M., 2009. Control of cell growth by the SCF and APC/C ubiquitin ligases. *Current opinion in cell biology*, 21(6), pp.816–24.

Smet, I. De et al., 2010. Bimodular auxin response controls organogenesis in Arabidopsis. *Proceedings of the National Academy of Sciences of the United States of America*, 107(6), p.2705.

Stockwell, B.R., 2004. Exploring biology with small organic molecules. *Nature*, 432(7019), pp.846–854.

Surpin, M. et al., 2005. The power of chemical genomics to study the link between endomembrane system components and the gravitropic response. *Proceedings of the National Academy of Sciences of the United States of America*, 102(13), pp.4902–7.

Surpin, M. & Raikhel, N., 2004. Plant cell biology: Traffic jams affect plant development and signal transduction. *Nature Reviews Molecular Cell Biology*, 5(2), pp.100–109.

Swarup, K. et al., 2008. The auxin influx carrier LAX3 promotes lateral root emergence. *Nature cell biology*, 10(8), pp.946–54.

Szemenyei, H., Hannon, M. & Long, J.A., 2008. TOPLESS mediates auxin-dependent transcriptional repression during Arabidopsis embryogenesis. *Science (New York, N.Y.)*, 319(5868), pp.1384–6.

Szyjanowicz, P.M.J. et al., 2004. The irregular xylem 2 mutant is an allele of korrigan that affects the secondary cell wall of Arabidopsis thaliana. *The Plant journal : for cell and molecular biology*, 37(5), pp.730–40.

Takeda, H., Farsiu, S. & Milanfar, P., 2007. Kernel Regression for Image Processing and Reconstruction. *IEEE Transactions on Image Processing*, 16(2), pp.349–366.

Tan, D.S., 2005. Diversity-oriented synthesis: exploring the intersections between chemistry and biology. *Nature Chemical Biology*, 1(2), pp.74–84.

Tan, X. et al., 2007. Mechanism of auxin perception by the TIR1 ubiquitin ligase. *Nature*, 446(7136), pp.640–5.

Templeman, W.G. & Marmoy, C.J., 1940. The effect upon the growth of plants of watering with solutions of plant-growth substances and of seed dressings containing these materials. *Annals of Applied Biology*, 27(4), pp.453–471.

The Arabidopsis Genome Initiative, 2000. Analysis of the genome sequence of the flowering plant Arabidopsisthaliana. *Nature*, 408(6814), pp.796–815.

Timpte, C. et al., 1995. The AXR1 and AUX1 genes of Arabidopsis function in separate auxin-response pathways. *The Plant Journal*, 8(4), pp.561–569.

Tiryaki, I. & Staswick, P.E., 2002. An Arabidopsis mutant defective in jasmonate response is allelic to the auxin-signaling mutant axr1. *Plant physiology*, 130(2), pp.887–94.

Tiwari, S.B., Hagen, G. & Guilfoyle, T.J., 2004. Aux/IAA Proteins Contain a Potent Transcriptional Repression Domain. *The Plant Cell*, 16(2), pp.533–543.

Tóth, R. et al., 2012. Prieurianin/endosidin 1 is an actin-stabilizing small molecule identified from a chemical genetic screen for circadian clock effectors in Arabidopsis thaliana. *The Plant journal : for cell and molecular biology*, 71(2), pp.338–52.

Tresch, S., 2013. Strategies and future trends to identify the mode of action of phytotoxic compounds. *Plant Science*, 212, pp.60–71.

Tsuda, E. et al., 2011. Alkoxy-auxins are selective inhibitors of auxin transport mediated by PIN, ABCB, and AUX1 transporters. *The Journal of biological chemistry*, 286(3), pp.2354–64.

Ulmasov, T. et al., 1997. Aux/IAA proteins repress expression of reporter genes containing natural and highly active synthetic auxin response elements. *The Plant cell*, 9(11), pp.1963–71.

Ulmasov, T. et al., 1995. Composite structure of auxin response elements. *The Plant cell*, 7(10), pp.1611–23.

Wagner, B.K. et al., 2008. Small-Molecule Fluorophores To Detect Cell-State Switching in the Context of High-Throughput Screening. *Journal of the American Chemical Society*, 130(13), pp.4208–4209.

Walsh, T.A. et al., 2006. Mutations in an auxin receptor homolog AFB5 and in SGT1b confer resistance to synthetic picolinate auxins and not to 2,4-dichlorophenoxyacetic acid or indole-3-acetic acid in Arabidopsis. *Plant physiology*, 142(2), pp.542–52.

Wassermann, A.M., Camargo, L.M. & Auld, D.S., 2014. Composition and applications of focus libraries to phenotypic assays. *Frontiers in Pharmacology*, 5, p.164.

Weigel, D., 2012. Natural Variation in Arabidopsis: From Molecular Genetics to Ecological Genomics. *PLANT PHYSIOLOGY*, 158(1), pp.2–22.

Weijers, D. et al., 2005. Developmental specificity of auxin response by pairs of ARF and Aux/IAA transcriptional regulators. *The EMBO journal*, 24(10), pp.1874–85.

Weijers, D. & Wagner, D., 2016. Transcriptional Responses to the Auxin Hormone. *Annual review of plant biology*.

Wen, R. et al., 2014. UBC13, an E2 enzyme for Lys63-linked ubiquitination, functions in root development by affecting auxin signaling and Aux/IAA protein stability. *The Plant Journal*, 80(3), pp.424–436.

Vernoux, T. et al., 2011. The auxin signalling network translates dynamic input into robust patterning at the shoot apex. *Molecular systems biology*, 7(1), p.508.

Vierstra, R.D., 2003. The ubiquitin/26S proteasome pathway, the complex last chapter in the life of many plant proteins. *Trends in plant science*, 8(3), pp.135–42.

Vieten, A. et al., 2005. Functional redundancy of PIN proteins is accompanied by auxin-dependent cross-regulation of PIN expression. *Development*, 132(20).

Wildman, S.G., 1997. The auxin-A, B enigma: scientific fraud or scientific ineptitude? *Plant Growth Regulation*, 22(1), pp.37–68.

Willige, B.C. & Chory, J., 2015. A current perspective on the role of AGCVIII kinases in PIN-mediated apical hook development. *Frontiers in plant science*, 6, p.767.

Wilson, A.K. et al., 1990. A dominant mutation in Arabidopsis confers resistance to auxin, ethylene and abscisic acid. *Molecular & general genetics : MGG*, 222(2–3), pp.377–83.

Woodward, A.W. & Bartel, B., 2005. Auxin: regulation, action, and interaction. *Annals of botany*, 95(5), pp.707–35.

Worley, C.K. et al., 2000. Degradation of Aux/IAA proteins is essential for normal auxin signalling. *The Plant Journal*, 21(6), pp.553–562.

Wu, M.-F. et al., 2015. Auxin-regulated chromatin switch directs acquisition of flower primordium founder fate. *eLife*, 4.

Wu, T.-C. et al., 2016. LobeFinder: A Convex Hull-Based Method for Quantitative Boundary Analyses of Lobed Plant Cells. *Plant physiology*, 171(4), pp.2331–42.

Xuan, W. et al., 2015. Root Cap-Derived Auxin Pre-patterns the Longitudinal Axis of the Arabidopsis Root. *Current Biology*, 25, pp.1381–1388.

Yamaguchi, N. et al., 2013. A Molecular Framework for Auxin-Mediated Initiation of Flower Primordia. *Developmental Cell*, 24(3), pp.271–282.

Yang, P. & Liu, K., 2015. Activity-Based Protein Profiling: Recent Advances in Probe Development and Applications. *ChemBioChem*, 16(5), pp.712–724.

Yang, X. et al., 2004. The IAA1 protein is encoded by AXR5 and is a substrate of SCF(TIR1). *The Plant journal : for cell and molecular biology*, 40(5), pp.772–82.

Zemlyanskaya, E. V. et al., 2016. Meta-analysis of transcriptome data identified TGTCNN motif variants associated with the response to plant hormone auxin in Arabidopsis thaliana L. *Journal of Bioinformatics and Computational Biology*, 14(2), p.1641009.

Zenser, N. et al., 2001. Auxin modulates the degradation rate of Aux/IAA proteins. *Proceedings of the National Academy of Sciences of the United States of America*, 98(20), pp.11795–800.

Zhu, J. et al., 2016. TWISTED DWARF1 Mediates the Action of Auxin Transport Inhibitors on Actin Cytoskeleton Dynamics. *The Plant cell*, 28(4), pp.930–48.

Ziegler, S. et al., 2013. Target Identification for Small Bioactive Molecules: Finding the Needle in the Haystack. *Angewandte Chemie International Edition*, 52(10), pp.2744–2792.

Zouhar, J., Hicks, G.R. & Raikhel, N. V, 2004. Sorting inhibitors (Sortins): Chemical compounds to study vacuolar sorting in Arabidopsis. *Proceedings of the National Academy of Sciences of the United States of America*, 101(25), pp.9497–501.

Acknowledgments

This chapter is concluding my PhD thesis and, I would like to express my grateful acknowledgments to the peoples and institutions who contributed in diverse ways to this work. Thanks to:

- Stéphanie Robert for giving me the wonderful opportunity to be her PhD student. I am deeply thankful for the project that challenged me every day and for your confidence in me.
- I would like to thank SLU/UPSC for being a wonderful research environment, the financial support from the Vetenskapsrådet and VINNOVA and the travel grants from the Kempe Foundation, EMBO and the Bröderna Edlunds foundation.
- Past and present Robert's group members for sharing all the challenges, I really enjoyed the supportive way we have been working together. A particular Thanks to Siamsa M Doyle, who took the time to correct all my texts with constructive comments and transformed my Frenglish into English.
- The collaborators I had the chance to work with.
- Jan Z., Aline V. and Samantha V. for critical reading of the thesis.
- Samantha V., Yohann B., Sylvain B., Julie T., Ludovic B., Delphine G., Mathieu C., Gaia G.C., Thomas S., Sylvie C., Ogonna O., Olivier K. and certainly peoples I have forgotten. You provided me friendly mentorship and you all contributed to help me concluding this chapter.
- Family and friends, deep thanks for making me who I am. Special thoughts to Ioanna and mon grand garçon.