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Decoding the auxin matrix: auxin biology through the eye of the computer

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ABSTRACT

The plant hormone auxin is certainly the most studied developmental regulator in plants. The many functions of auxin during development, from the embryo to the root and shoot construction, are mediated by an ever-growing collection of molecular regulators, with an overwhelming degree of both ubiquity and complexity that we are still far from fully understanding and that biological experiments cannot grasp alone. In this review, we discuss how bioinformatics and computational modelling approaches have helped in the recent years to explore this complexity and to push the frontiers of our understanding of auxin biology. We focus on how analysis of massive amounts of genomic data and construction of computational models to simulate auxin-regulated processes at different scales have complemented wet experiments to understand how auxin acts in the nucleus to regulate transcription and how auxin movement between cells regulates development at the tissue scale.

Key words

Auxin signaling, polar auxin transport, development, evolution, bioinformatics, computational modeling, synthetic biology

INTRODUCTION

Auxin is one of the phytohormones that act in coordinating plant development with internal and external signals. Its history goes back in time to the 18th century when Charles Darwin and his son, Francis, proposed the existence of a mobile signal whose distribution led to differential growth rates in grass coleoptiles, inducing a curvature towards the light. Although they did not know at that time that this mobile signal was auxin (it took 50 more years to identify it as such) these

experiments were already pointing out two key characteristics of the auxin molecule: its involvement in plant growth regulation and the importance of its movement to carry out its function.

Regarding the regulation of plant growth, since the Darwins, we have found that auxin does much more than just pointing plants towards light. All along a plant life and throughout a plant body, there is a role for auxin: axes determination in the embryo, formation of lateral roots or determining the position of the flowers at the shoot tip are just but a few examples. These effects, visible at the organ or full-plant scale, rely on processes at the cell scale, where the auxin signal is processed and translated into transcriptional responses but also rapid non-genomic responses that allow auxin to control developmental programs.

As for auxin movement, auxin travels through diffusion or in a non-directional manner in plant vascular tissues, as other hormones do. However, auxin is also transported directionally from cell-to-cell. This active transport, known as polar auxin transport, is exclusive to auxin and creates differential auxin distribution within tissues that are fundamental for many plant developmental processes (11, 134). A variety of polar and non-polar auxin transporters located at the plasma membrane but also others located in intracellular membranes regulate this cell-to-cell directional auxin movement, as well as compartmentalization of auxin thus controlling hormone distribution within cells.

During the past decades, experimental biology has allowed us to identify and better understand the molecular determinants of auxin signaling and movement. At the same time, the large collection of molecular regulators identified has revealed an overwhelming degree of both ubiquity and complexity in the way auxin regulates plant development that we are still far from fully understanding and that biological experiments cannot grasp alone. Computational modeling has

allowed to start exploring this complexity as well as predicting and testing *in silico* novel biological properties expanding significantly our knowledge on auxin biology. At the same time, rapid progresses in genomic technologies have also allowed generating massive amounts of sequence data from a diversity of plants, providing a full new dimension to our understanding of how auxin functions. Here, we review how computer-based approaches, namely computational biology and bioinformatics, have helped pushing the frontier of our understanding of auxin biology, focusing on its action in the nucleus to regulate transcription and on its movement between cells to regulate development at the tissue scale.

1. Processing auxin information in the nucleus: from perception to gene regulation

1.1. A trio of components controlling auxin-induced transcriptional responses

As mentioned previously, auxin perception in a cell triggers non-genomic responses (See (34) for review) but also massive transcriptional changes that will be our focus here. These changes are controlled by the Nuclear Auxin Pathway (NAP), with three families of proteins that perceive, transduce, and transcribe the auxin signal into a transcriptional output. These are the TRANSPORT INHIBITOR RESPONSE1/AUXIN-SIGNALING F-BOX (TIR1/AFB) receptors, the Auxin/indole-3-acetic acid (Aux/IAA) repressors and the Auxin Response Factors (ARF) Transcription Factors (TFs). ARFs are divided in three evolutive clades, A, B and C, and can be transcriptional activators (Clade A) or repressors (clade B and C) (40). Interactions between these proteins drive auxin-induced transcriptional responses. The best-described model that explains this transcriptional regulation is based on a de-repression mechanism (76): Aux/IAAs maintain auxin signaling repressed through their interaction with ARFs, blocking their action. An increase in auxin levels triggers the formation of a co-receptor complex between TIR1/AFB receptors and Aux/IAA

that leads to Aux/IAAs degradation. Once the repressors are degraded, ARFs are able to induce expression of auxin responsive genes (Figure 1, upper left panel).

Numerous studies, mostly done with the model plant *Arabidopsis thaliana*, have helped to establish this scenario. However, this model is far from being complete. 6 TIR1/AFBs, 29 Aux/IAAs and 23 ARFs constitute the *A.thaliana* NAP. Out of the 23 ARFs, the model described above only applies to 5, the activators or clade A ARFs. Most clade B and C ARFs lack the capacity to interact with a large number of Aux/IAAs and therefore they must be connected to auxin signaling through other mechanisms, still under exploration.

ARFs differential capacity to interact with Aux/IAAs and thus, to respond to auxin is only an example of the important diversity of molecular properties and mechanisms potentially hidden within the NAP pathway. Additionally, NAP members have different expression patterns in the plant (114, 139). Altogether, this creates a scenario where different cells or group of cells, depending on the TIR1/AFB-Aux/IAA-ARF cocktail present in them and the biochemical properties of these elements could translate a same auxin input into completely different outputs. By playing with combinations of NAP elements and their properties, auxin has the potential to generate thousands of different responses, which could explain the diversity, specificity, and complexity of the developmental programs it regulates. Below, we will see, first, how omics approaches helped to understand how this diversification and complexity evolved. Then, we will review how computational biology has permitted to better grasp the complexity of the NAP through the use of mathematical models.

1.2. Evolution of the NAP: big families have complex issues

The scenario presented for *A. thaliana* and its inherent complexity is representative of the situation in Angiosperms. Recent phylogenetic studies showed the existence of a simpler, yet conserved, NAP in bryophytes, a clade located at the beginning of plants evolutionary history (18, 40). But was auxin also able to regulate transcription before plants transitioned to land? Due to the lack of large-scale data from plant aquatic ancestors, green algae, this question remained unanswered for a long time. This changed thanks to the 1000 Plants Initiative, or 1KP project, that generated a large transcriptomic dataset from more than 1000 species of land plants and green algae, providing the scaffolds for wider plant phylogenomics (23).

Several studies made use of this invaluable tool to look for traces of the NAP in green algae. These studies identified the existence of two clades of ARF charophycean homologues: protoARF-A/B and protoARF-C (Figure 1). According to residues conservation, structure modelling and biochemical experiments, these protoARFs already possess the capacity to bind ARF binding sites (41, 45, 83, 99). On the contrary, TIR1/AFB and Aux/IAA charophyte ancestors lacked the residues or domains necessary to interact with auxin and between each other. Altogether, these studies suggest the existence of an ancient auxin-independent transcriptional regulation relying on ARF TFs (Figure 1). Gain of the TIR1-Aux/IAA co-receptor complex and Aux/IAA-ARFs interaction could have permitted the co-option of this regulatory system into the NAP, turning ARF-regulated genes into auxin-responsive genes (41, 83, 99).

Although very informative, the use of transcriptomic data for evolutionary studies can introduce biases since certain genes might not be detected if repressed under experimental conditions. Some charophyte genomes have recently been sequenced (56, 101, 143) and the sequel of the 1KP project, the 10KP project (24), aims at sequencing genomes from over 10000 representatives from

different plant clades. These genomic data will provide a more reliable genomic platform for future auxin evolutionary studies.

Indeed, a fundamental event in the understanding of auxin signaling evolution was the whole genome sequencing of the liverwort *Marchantia polymorpha*, that revealed the existence of a complete and functional NAP in this organism composed of only 1 TIR1/AFB, 1 Aux/IAA and 3 ARF genes, divided already in three evolutive clades: A and B, derived from a duplication event in the A/B charophycean clade, and clade C (41, 99) (Figure 1). Physiological studies showed that the molecular functions and developmental roles of *Marchantia* NAP components are highly conserved across land plants (59, 60). Therefore, sequencing of *Marchantia*'s genome permitted to conclude that TIR1/AFB-Aux/IAA and Aux/IAA-ARF interaction modules, missing in charophytes, are a land plants innovation and allowed to finally position the origins of the NAP in the last common ancestor of land plants.

Combination of genomic and transcriptomic data from several plant species have allowed to identify duplication events in all three families of proteins that led to a massive expansion of the NAP in higher plants (40, 41, 99). Evolutionary innovations arose from such duplications generating the molecular and biochemical diversity that characterizes the NAP.

1.3. Bioinformatics-based exploration of auxin response specificity: the ARF-DNA reading code at the genome-wide scale

The ultimate control of auxin transcriptional responses relies on ARF transcription factors. Where on the genome they are bound will determine which genes will be induced or repressed in response to auxin. ARFs bind to TGTCnn consensus sequences, named Auxin Response Elements (AuxRE), located in promoters of auxin responsive genes. Biochemical, structural and synthetic biology

studies using reconstruction in orthologous organisms showed that different ARFs have different preferences for AuxRE sequences (15, 112) pointing at the ARF-DNA interface as a likely source of diversity and specificity of the auxin transcriptional response. In the last decade, transcriptomic and genomic data combined with more and more powerful bioinformatics tools have permitted to study ARF-DNA interactions at the genome-wide scale.

Transcriptomic data of gene expression in response to auxin showed that differences in the TGTCnn sequence or orientation are associated to up or down-regulation of genes in response to auxin (42, 129, 148). TGTCnn elements are also found frequently associated to *cis* elements for other TF families suggesting the action of co-factors and hormonal cross-talk with auxin responses (12, 25, 103).

Genomic data for ARF binding are available thanks to the recently developed DNA Affinity purification sequencing (DAP-seq) technique (Sidebar 1). DAP-seq uses TF-DNA complexes with recombinant TFs and fragmented naked genomes to identify TF target regions *in vitro* (9, 104). DAP-seq was applied to ARF TFs from clades A and B from *A. thaliana* and maize and it showed that different clades of ARFs do not bind the same regions in the genome. This specificity seems to rely on their binding as dimers to double AuxREs with two parameters, orientation and spacing between two AuxREs, being fundamental determinants of ARF DNA binding preferences (43, 104, 129) (Figure 2.A). Separation of A and B clades in land plants must have come with a diversification of their DNA binding properties. On the contrary, ARFs from a same clade share similar DNA binding preferences (43) suggesting that these have been conserved within each clade along evolution. Certain genes are bound by both ARF clades (43, 129) pointing at a potential mechanism of competition between ARFs a theory supported by *in vitro* experimental data: Kato *et al*. showed that different ARFs have constant affinities for DNA within the same nanomolar

range, a theory compatible with the possibility of a competition (60). Competition for binding to DNA is indeed one of the hypotheses that could explain how repressor ARFs regulates auxin-induced transcriptional responses (see below).

A similar analysis using Chromatin Immunoprecipitation Sequencing (ChIP-seq) data (Sidebar 1) yielded similar results about *A. thaliana* ARF6 (clade A) binding *in vivo* (103) that those obtained for clade A ARFs using DAP-seq. On the contrary, *A. thaliana* ARF3 ChIP-seq data yielded several novel configurations of ARF binding sites not detected in DAP-seq data sets (129). ARF3 is a clade B ARF but with special properties since it directly binds auxin and lacks the ARF C-terminal PB1 domain that mediates interaction with Aux/IAAs (125, 126). These results suggest that ARF3 might have a different binding behavior than other clade B ARFs.

Altogether, transcriptomic and ARF genomic binding data suggest that indeed, the sequence of AuxREs and ARF differential binding properties constitute an essential element of specificity in the regulation of auxin transcriptional responses. Still, ARF genome-wide binding remains largely unexplored *in vivo*. ChIP-seq data is only available for *A. thaliana* ARF6 and ARF3. No genome-wide binding information exists for clade C proteins. Clade C ARFs present a different dimerization interface in their DBD that suggests that they might have a different binding behavior (83). Additionally, the role of this clade in the NAP is currently under question. Although initially classed as repressor ARFs, recent studies question whether clade C ARFs are even linked to auxin signaling (41, 60). Genome-wide information about the regions bound by clade C ARFs might shed some light as to whether these proteins can regulate auxin-responsive genes.

1.4. Mathematical models and synthetic biology to unravel auxin signaling complexity

Molecular modules acting upstream of ARF-DNA interaction also contribute to expand diversity in auxin transcriptomic responses: TIR1/AFB and Aux/IAA proteins possess different affinities for auxin and for each other (22), Aux/IAAs have different degradation rates and some are non-degradable by auxin (94), and as previously mentioned, a fundamental determinant for ARF mechanism of action is ARF-Aux/IAA interactions that are different depending on the ARF (139). Systems biology allows, through the use of computational models (Sidebar 2 and 3), to simulate the behavior of the system, and to raise and test hypotheses as to how each of these modules and their diversity contribute to build up auxin responses.

One of the main open questions about auxin signaling is how repressor ARFs take part in the whole process. Although several hypotheses have been proposed based on experimental evidences (TPL recruitment or competition for DNA that we have mentioned in section 1.3) (26, 73) there is no general mechanism of action described for these ARFs. A high throughput yeast two-hybrid analysis of the ARF-Aux/IAA interactome showed that – with some exceptions – clade B and C ARFs are not able to interact with Aux/IAAs (Figure 2.B). A mathematical model based on these experimental data predicted an important role for repressor ARF (clade B and possibly clade C ARF) in determining auxin sensitivity of a tissue and in buffering auxin signaling, conferring robustness to the pathway upon auxin level variations (139). Clade A, B and C ARFs are co-expressed in the shoot apical meristem (SAM) of *A. thaliana* (with differential expression between the center and the peripheral zone of the SAM) and live-imaging using two complementary auxin sensors, DII-VENUS to visualize auxin levels (20, 139) and DR5 to measure auxin signaling activity, validated the predictions in the SAM (Figure 2.B). In *Marchantia*, imaging of translational fusions of two ARFs, an activator and a repressor, identified zones with different activator:repressor stoichiometries, pointing towards the same idea that repressor:activator ARF

combinations delimit zones of sensitivity to auxin. The simplicity of *Marchantia* NAP indeed makes of this plant an ideal tool to test predictions from computational models (60).

An interesting approach done in Vernoux *et al*. (139) is the use of clustering as a way of simplifying a complex interaction network. It identified clusters of components with similar biological properties (in this case protein-protein interaction properties) that can be considered as single abstract entities in the model, here activator ARFs interacting with Aux/AA, Aux/IAA interacting with themselves and with activator ARFs, and repressor ARFs not interacting with the others. Further models predicted that ARF:Aux/IAA, Aux/IAA:Aux/IAA and ARF:ARF modules control differently the amplitude, speed and sensitivity of the response when considering class A ARFs (37), thus potentially explaining how the diversification of the families class A ARFs and Aux/IAA has expanded the regulatory potential of this signaling pathway (Figure 2.B).

Other ARF-Aux/IAA interactomes have confirmed the results of (139) but also showed that certain ARFs and Aux/IAAs behave differently (113, 139). Indeed, when including protein sequence information to identify clusters in ARF-Aux/IAA interactome, an outlier cluster with different interaction properties emerged. This cluster contained ARF9 and 22 (clade B repressors) and Aux/IAA 32, 33, 20 and 30 (74). ARF9 interacts with most Aux/IAAs, similarly to activator ARFs (139). Whether this property confers ARF9 an auxin-dependent mechanism of action despite its predicted repressor role remains to be studied. On the other hand, Aux/IAAs of the outlier cluster correspond to non-canonical Aux/IAAs that lack the degron motif, the domain that mediates interaction with TIR/AFB and degradation in response to auxin. Clade C ARFs have been shown to interact with certain non-canonical Aux/IAAs (113). This interaction has been proposed as an additional mechanism of repression of auxin transcriptional responses (80). ARF and Aux/IAA homo and hetero-interactions happen through a shared C-terminal PB1 domain. Differences in

PB1 residues composition determine how ARF and Aux/IAA interact. Structural data have permitted to identify the residues implicated in these interactions and has helped to better understand how these complexes are formed (62, 65, 100). However, PB1 structures for clade B and C ARFs are missing and we can only speculate about how these ARFs establish interactions (106). Interestingly, the clustering approach suggests that the more different PB1 sequences are the stronger their interaction is (74). This can help predicting interactions for NAP from other plants and have a better understanding of how the ARF-Aux/IAA module evolved.

As it happens with the PB1 domain, Aux/IAAs differ in degron residue composition and have different degradation rates (94). Combination of computational modeling and synthetic biology in yeast has permitted to quantify these degradation rates (52) and to predict how variations in this parameter affect auxin responses (111). A minimal Auxin Response Circuit (ARC) including one AFB, one Aux/IAA, one ARF, one auxin-responsive promoter and TPL co-repressor can recapitulate auxin-induced transcription in yeast (Figure 2.C). Using different Aux/IAA members to construct an ARC results in different dynamics of the transcriptional response. Mathematical models predict Aux/IAA degradation rates as the main parameter influencing these changes. Therefore, natural variations in Aux/IAA degradation rates caused by different degron residue composition deeply affect the dynamics of the auxin response (111) (Figure 2.C).

Several family members frequently control simultaneously the same process (114, 139). Building a model with two Aux/IAAs and two ARFs correctly predicts Aux/IAA3 and 17 opposite roles in the formation of root hairs and lead to hypothesize Aux/IAA competition as the molecular mechanism controlling this process (19). Indeed, including two Aux/IAAs in the previously explained ARC reconstructed in yeast retrieved auxin dynamics closer to the one observed with one of the two Aux/IAAs (111), a result that supports the idea of competition, with one of the two

proteins having a dominant role. This experiment was done with four different Aux/IAAs co-expressed during LR formation that have sequential roles in this process. Interestingly, the dominance hierarchy observed in yeast matches their sequential roles (111) (Figure 2.D) Competition between non-canonical and canonical Aux/IAAs for the interaction with clade C ARFs has been demonstrated experimentally. Binding of non-canonical Aux/IAAs prevents ARFs from being blocked by canonical Aux/IAAs and constitutes and additional mechanism of regulation of auxin responses (80).

Several Aux/IAA genes are ARF targets and their expression is rapidly induced in response to auxin. This creates a negative feedback loop, with newly synthesized Aux/IAA proteins repressing auxin signaling. Mathematical models predict that this feedback loop can generate oscillatory auxin responses, with Aux/IAA mRNA/protein ratio being fundamental to control the dynamics of such responses (89). Oscillatory auxin responses are involved in Lateral Root (LR) formation. LRs are primed in the root pericycle in the transition zone of the root meristem. Priming follows an oscillatory behavior along the root and involves changes in auxin-triggered gene expression. Notably, DR5 auxin reporter expression happens in waves that precede LR branching sites (92). Such patterns could be explained by an oscillatory circuit like the one predicted in (71, 89). By a combination of experimental data and computational modeling ARF7 and Aux/IAA18 were identified as the two components establishing the auxin-dependent negative feedback loop that controls LR formation. Modeling of such circuit correctly predicts waves of DR5 in pre-branch sites (Figure 2.E), in line with the previous hypotheses (108).

Following LR priming, DR5 expression remains stable in the pre-branch sites. Considering that auxin transport is highly active in the root tip (see section 2), it is unlikely that the DR5 stable signal is driven by a constant auxin maximum. DR5 stable expression could then result from a

memory mechanism, allowing cells to continue the LR formation program even if auxin signal is not there. Memory patterns can be explained by switch circuits that generate bi-stable systems (71). Lau *et al.* described such a system for the BODENLOS /MP (Aux/IAA12/ARF5) interacting partners. A synthetic biology approach using protoplasts revealed that apart from the negative feedback loop where MP induces BDL and BDL represses auxin signaling, MP is also able to induce its own expression creating a positive feedback loop. Computational modeling predicts that this circuit generates a bi-stable system: an increase in auxin levels leads to a steady state for MP and BDL and, after decrease in auxin levels, MP-controlled genes can remain up-regulated several hours (72). Other ARF-Aux/IAA combinations could establish similar circuits that could provide a memory of the auxin signal after LR priming (Figure 2.E).

Both computational and synthetic biology have thus evidenced the importance of controlling co-expression of components and interacting partners in auxin signaling. Computational modelling has permitted to predict additional mechanisms of auxin responses regulation and to understand how the diversity of NAP components helps controlling the complexity of auxin responses. Synthetic biology in yeast and protoplasts has revealed itself as an invaluable tool to complement computational biology studies. Single cell RNA-sequencing (scRNA-seq) is rapidly developing in plants and scRNA-seq has been successfully applied to *A. thaliana* roots, shoot and vegetative meristems and maize SAM (4, 123, 149). In the future, this technology should allow further identifying co-expressed NAP components with a single cell resolution, allowing together with computational models and synthetic biology to further understand the combinatorial properties of the NAP in various plants.

2. Multiscale regulation of auxin spatio-temporal distribution during plant development

Auxin travels within the plant but also within cells to reach nuclei to activate transcriptional responses, and thus act at multiple scales. In this section, we discuss the contribution of bioinformatics and computational modeling (Sidebar 2 and 3) to our knowledge on the control of auxin spatio-temporal distribution at the tissular and cellular scale during development.

2.1. The molecular actors of Auxin transport and their evolution

Auxin gradients constitute a common regulatory module for plant development (11, 134). As with other plant hormones, auxin can first be transported passively, through diffusion across the plasma membrane (PM; mostly from the apoplast to the cytoplasm given auxin chemical properties), in the apoplast or in the symplasm through plasmodesmata. Transport through plasmodesmata is regulated by their permeability, that depends on callose deposition (for review (78)) (Figure 3). Callose-mediated control on auxin diffusion through plasmodesmata has been proven important in *A. thaliana* and *P. patens* for several developmental processes (28, 51, 121). In addition to these mostly passive regulations, the generation of auxin gradients involves an active directional cell-to-cell transport driven by plasma membrane (PM) transporters (110). Four main families of PM transporters drive auxin in and out cells: the PIN-formed (PIN), PIN-like (PILS), AUXIN1/LIKE-AUX1 (AUX1/LAX) and ATP-BINDING CASSETTE B4 (ABCB) proteins. As for auxin signaling, these proteins are encoded in angiosperms by multigene families with different properties. However, the molecular mechanism allowing them to transport auxin are not as deeply understood as those involving auxin signaling components.

PIN proteins were the first molecularly characterized auxin transporters. PINs function as efflux carriers and are classified as canonical and non-canonical. Canonical PINs (e.g. PIN1,2,3,4,7 in Arabidopsis) are polarly-localized at the PM and transport auxin from cell to cell, whereas non-

canonical PINs (e.g. PIN5,8 in Arabidopsis) localize to the Endoplasmic Reticulum (ER) membrane (Figure 3). Both classes share a structure with 10 transmembrane domains separated by a hydrophilic loop. PIN polar localization in the membrane involves regulation of PIN expression, protein recycling and clustering (Reviewed in (3)). Internal and external signals such as hormones (auxin or others) or mechanical stresses can alter PIN presence on the membrane (53, 109, 133) and therefore, auxin transport. The members of the second and more recently discovered family of auxin transporters, the PILS, are also localized at the ER membrane and have a structure similar to non-canonical PINs (8, 39). Finally, AUX1/LAX and ABCB auxin transporters regulate auxin influx and influx/efflux respectively and are part of larger families of transporters (Figure 3) (47, 132).

Availability of genomic and transcriptomic data has allowed to trace the history of some of these auxin transporters along plants evolution. Contrary to auxin signaling, that was a land plant innovation, auxin transport seems more ancient. Homologues of the four protein families are present in chlorophyte and charophyte lineages (31, 39, 56, 101, 140, 143), although secondary losses have taken place in some charophyte clades (140). Due to the lack of more diverse chlorophyte genomic information, we cannot fully reconstruct the evolutionary history of these proteins for now but available data suggest that transport of auxin predates the emergence of auxin signaling. Despite their similar predicted proteins structure and functions, ER-localized PINs and PILS could have evolved independently (39). However, bacterial transporters that also possess a ten transmembrane-domain topology have been identified as PIN homologues. Evolutionary trees including these transporters suggest a possible common origin of PILS and PIN from bacterial transporters (16). In single-cell organisms, these proteins could be acting as auxin exporters to the medium, either as a detoxification mechanism –auxin has adverse effects on some algae- or as a

communication signal with the environment (140). On the contrary, in the charophyte algae *Chara corallina* evidences exist for polar auxin transport from apex to rhizoids (17). Therefore, the set of charophycean auxin transporters have the potential to trigger a plant-like auxin transport in multicellular algae with more complex body structures. Recent additions to auxin transporters are WALLS ARE THIN 1 (WAT1) – auxin exporter from the vacuole- or the NITRATE TRANSPORTER 1.1 (NRT1.1) with a dual auxin/nitrate transport function (67, 115). These are part of other protein families present in streptophyte clades but for which there is no evidence of their role as auxin transporters in algae and these could have evolved a substrate specificity for auxin later in evolution (16).

2.2. Auxin movement within the cell

Conservation of ER-localized PIN and PILS transporters from chlorophytes to angiosperms suggests the existence of an intracellular auxin transport regulation. In the absence of knowledge on auxin intracellular distribution, modeling offers a powerful way to test this hypothesis. Computational models including non-canonical PIN auxin transport from the cytoplasm to the ER (141) or from the ER to the nucleus (88) suggest that the ER acts most certainly as a regulatory platform to control auxin intracellular distribution. In accordance with wet experiments performed in protoplasts, the ER appears notably to act as a conduit that exports auxin to the nucleus, a mechanism that could be key to control auxin concentration in the nucleus and auxin transcriptional responses (88) (Figure 4.a). An analysis of the distribution of auxin within the cell, e.g. using the recent AuxSen auxin FRET sensor (54), could allow to test further this regulatory mechanism in the future. Other intracellular compartments could also contribute to regulate nuclear auxin concentration, for example the vacuole through WAT1. Importantly,

knowing more about auxin intracellular distribution mechanisms will also likely be essential to understand fast non-nuclear auxin responses, as TIR1/AFB co-receptors have been demonstrated to be involved in some of these responses (33, 38) and this could depend on auxin availability in the cytoplasm.

2.3. Understanding auxin polar transport at the cellular and tissular scale: an ongoing quest

As previously mentioned, PIN endosomal recycling, regulated by a large number of molecular actors, plays an essential role in establishing PIN polarities within a cell (3). Super-resolution imaging and *in silico* analyses identified polar delivery by exocytosis to the center of the polar domain of the plasma membrane as essential to establish polarity, while PIN recruitment to non-mobile membrane clusters limits PIN diffusion and endocytosis at the edge of the polar domain regulate the maintenance of polarity of the PIN transporters (63, 70). Other regulations are likely at play, such as the composition of the cell wall, cell wall-plasma membrane connections or the cytoskeleton (70) that could be involved in PIN clusters formation, several kinases that can phosphorylate PINs on various residues (69) or mechanical forces (53) that can affect PIN polarity. Incorporating these regulations into novel computational models will certainly not only help but also be necessary to disentangle how the multiple regulations involved act in synergy to regulate PIN polarity at the cells scale.

At the tissue scale, modeling approaches using different hypotheses have been key to tackle the essential question of the emergence of PIN polarity distribution during development. The flux-based or canalization hypothesis was originally proposed to explain mid-vein formation (120). With our current knowledge, this hypothesis proposes that PIN localization at a membrane is increased by the auxin flux going through it, thus stabilizing the auxin efflux. The flux-based hypothesis has been used to explain the emergence of PIN polarities not only in veins (10) but also

during embryogenesis (142), phyllotaxis at the shoot apical meristem (130) and leaf outgrowth (2) (Figure 4.b). The concentration-based hypothesis has been formulated first to explain PIN-based phyllotactic patterning at the shoot apical meristem, with auxin accumulation at sites of organogenesis together with concomitant auxin depletion around the organ (58, 116, 127). This hypothesis postulates that PIN1 polarity in a given cell is established toward the neighboring cell with the highest auxin concentration, resulting in auxin accumulation at PIN convergence sites in the shoot apical meristem (58, 127), during leaf margin formation (14, 61) and during the dynamic formation of ovules (145). A model using the concentration-based hypothesis could in theory also explain vein formation (87) (Figure 4.b). Concentration- and flux-based mechanism can also be combined in a single tissue and this dual-polarization approach has been used to reconcile organ site specification and the formation of associated vascular tissue in the shoot apical meristem (10, 102).

While conceptually different, the flux-based and concentration-based hypotheses both rely on the idea that auxin positively feedbacks on its own transport. Such feedback regulation provides a plausible explanation to PIN polarity coordination and auxin distribution self-organization during development. Auxin affects PIN endocytic trafficking, although how this occurs molecularly is still debated (for review and discussion:(133)). Several regulators of PIN intracellular trafficking affecting auxin-dependent cellular polarity and coordination of PIN polarities, have been recently identified (49, 50), laying the ground for understanding precisely the mechanisms involved. Auxin signaling components (13), several downstream targets of auxin (81, 122) and mechanical signals (53, 82) provide extra layers of cell autonomous and non-cell autonomous regulations of PIN polarity that have been tested using computational models. However, all these mechanisms remain largely uncharacterized at the moment and how the known molecular mechanisms of polar auxin

transport could explain how a cell senses auxin fluxes in membranes or auxin concentration in neighboring cells as postulated for the flux-based or concentration-based hypotheses remains unanswered so far. In addition, Abley et al. (1, 2) proposed an alternative model assuming that cells are inherently polarized and thus that polarization does not require external asymmetries in auxin concentration. Polarization at the tissue-scale then results in this model from a hypothetical mechanism coordinating individual polarities. Comparison of models (2, 135) suggest that all models have strength and limitations. Further biological data on the mechanisms of PIN polarity regulation, on auxin spatio-temporal distribution, as e.g. in a recent report in the shoot apical meristem (44), as well as parameter measurements (Sidebar 3) will thus be required in the future to fully understand how polar auxin transport coordination is achieved at the tissue scale and whether the existing models capture the properties of the mechanisms at play.

2.4. Shaping auxin distribution during plant development: PIN rules but not alone

In addition to highlighting the central role of PIN proteins, modeling approaches have been instrumental to establish that, despite the central role of PIN polarity distribution discussed above, in many cases additional regulations are required to establish the observed auxin distribution and the subsequent dynamic development of the tissues. We illustrate here with chosen examples how other molecular mechanisms act in synergy with auxin efflux and refer the reader to recent reviews for further discussion (27, 119).

In most models analyzing either the emergence of PIN polarity patterns or the capacity of a distribution of polarity to generate an auxin distribution, the focus has been on polarity considering an identical quantity of PIN proteins in all cells of the tissue. However, differential expression of PINs can also contribute to control auxin distribution. PIN3, PIN4 and PIN7 are

required for generating an auxin differential between the convex and concave side of hypocotyl that triggers the formation of the Arabidopsis apical hook after germination (146). Analysis of the expression of the efflux carriers and a computational model demonstrated that a differential accumulation of PIN4 and PIN7 in both the epidermis and cortex on the hook convex side is required (147), in contrast to previously suggested differences in the cortex only (146). Another example occurs in the valve during fruit maturation where PIN3 differential expression and an apolar localization in the valve is predicted to allow for the formation of a minimum in the tissue, a process involved in the ripening of the fruit (77). These studies and the fact that most *PIN* genes are regulated by auxin through a mechanism that involves at least in part ARFs (66) suggest that differential expression of PINs could be an essential factor for the regulation of auxin distribution dynamics that should be considered more extensively in the future.

ABCB proteins have been suggested to be either efflux or influx non-polar carriers and to interact with PINs for the regulation of auxin transport through hypothetical mechanisms (For review: (47)). However, their contribution to the regulation of auxin distribution remains unclear. Mellor et al. (85) combined imaging, genetics and a model incorporating ABCBs and their differential distribution to analyze ABCB function in controlling auxin distribution in the root tip. They demonstrate that ABCBs are required for proper auxin distribution measured with the DII-VENUS biosensor (20), and provide the first evidence that all the ABCBs in the root act in regulating efflux, that they can fulfill this function independently of PINs and that PIN-mediated efflux is co-dependent on ABCBs where the proteins are co-expressed (Figure 4.c). Modeling has similarly been key to establish how influx regulated by differentially-expressed AUX1/LAX carriers acts with PINs in the regulation of auxin distribution in various tissues. Incorporating AUX1/LAX differential regulation of influx in a root model is required to obtain more realistic

auxin distributions visualized with DII-VENUS (20) and suggests that AUX1/LAX mediated influx controls which tissues have high auxin (6) (Figure 4.c). Still in the root, a computational model and mutant analysis shows that expression of AUX1 specifically in the protophloem cell files within the vasculature creates a lateral inhibition that is required for differentiation into sieve elements when auxin efflux is perturbed (93), a mechanism that could provide robustness to this differentiation process. A differential activity of AUX1/LAX proteins is also required to obtain periodic vascular bundles formation in the inflorescence stem (36). In addition, auxin transport mediated by carriers is not sufficient to control the root auxin distribution fluxes. Computational modeling taking into account differential plasmodesmata density distribution (150) and manipulation of plasmodesmata opening shows that diffusion of auxin through plasmodesmata is required for the auxin reflux loop between the outer and inner root tissues that allows to maintain high auxin levels in the root meristem (48, 86) (Figure 4.c). Gao *et al*. (46) further demonstrated with a combination of auxin diffusion quantification and modeling that plasmodesmata distribution in the leaf allows to channel auxin diffusion along the midrib. Together with previous demonstration that auxin distribution is perturbed in *Arabidopsis* mutants affected in plasmodesmata activity (For review: (5)) but also in moss (28), these studies shows that symplastic diffusion regulates auxin movement within tissues.

Spatial control of auxin biosynthesis is also an essential parameter in the control of auxin tissular distribution. Using a canalization model, the early specification of sites of auxin biosynthesis has been proposed to organize PIN polarities and thus auxin distribution within the embryo (142), coherently with the embryonic phenotype observed in *yucca* (*yuc*) mutants affected in auxin biosynthesis (118). Mapping of *YUC* and *TAA1/TAR* auxin biosynthesis genes and a canalization model suggest that localized auxin biosynthesis is also a prerequisite for vasculature

development in the developing leaf (64). In the root, auxin coming from the shoot is used in models as the major auxin source in the root meristem (6, 48). However, an enrichment in auxin biosynthesis enzymes is seen in and around the quiescent center and this expression has been proposed to be required for root meristem maintenance (128). A higher auxin production around the quiescent center is postulated in most root models (e.g. (6, 32, 86, 122)) and a theoretical approach showed that this local biosynthesis, together with the auxin reflux loop, could maintain auxin levels in the root and sustain growth in the absence of a shoot-derived auxin source (82). Experimental evidence show that auxin biosynthesis is also essential for shoot meristem function and phyllotaxis (44). Shi *et al.* (124) used a flux-based auxin transport model, imaging and genetics to show that auxin transport from lateral organs regulate auxin levels in the SAM and the size of the stem cell niche. Lateral organs are predicted to be sites of auxin biosynthesis in the shoot meristem (44) but how this impacts auxin distribution and meristem function remains to be analyzed in more details notably by incorporating this regulation in computational models. Finally, auxin inactivation by GRETCHENHAGEN3.17 (GH3.17)-mediated conjugation in the outermost columella cells, lateral root cap and to a lower level in the differentiated epidermis has been shown to be required to generate a local minimum of auxin positioning the boundary between proliferating meristematic cells and differentiating cells (32, 122).

Thus, studies based on computational modeling approaches have helped positioning a plethora of mechanisms acting in synergy with PINs to regulate auxin distribution and auxin-mediated developmental processes. This highlights a regulatory framework of an exquisite complexity. Future modeling work will undoubtedly be required to understand the respective contribution of these mechanisms and of the many feedbacks between them, and to cast light on how auxin achieves its many developmental functions.

3. Integrating auxin with other signals

Auxin is of course not fulfilling its functions in isolation but rather interacts with many internal and external signals, and these interactions are key for setting and/or for the developmental outcome of a given auxin distribution. Understanding signal integration during development is challenging and we highlight here examples where computational models have been instrumental to explore the mechanisms involved.

3.1 Auxin cross-talks with many hormones and other developmental mechanisms

Auxin and cytokinins (CKs) have mostly antagonistic roles at the heart of many developmental processes. Several key nodes have been identified in the auxin-CK crosstalk: CKs can induce auxin biosynthesis and vice versa, ARFs bind and regulate the expression of CK signaling effectors, and CK controls auxin transport altering expression of PINs (For Review: (84)). Computational models have explored the role of these regulatory nodes both at the cell and multicellular scales, supporting essential contribution to specifying the balance between proliferation, elongation and differentiation in the root meristem (32, 96), lateral root founder cell specification (98), periodic lateral root branching (95) and in vascular patterning by transcription factors and microRNAs (30, 35, 85, 97). Ethylene influences PIN and AUX1 expression and together with CKs is also required to predict auxin patterns in an *Arabidopsis* root cellular model (79, 90, 91). GAs promotes cell proliferation during the early stages of root development and a computational model allowed concluding that GAs are required with auxin and CKs to determine cell behaviors within the meristem, with a prediction that levels of the GA repressor RGA emerge from an auxin control on GA synthesis (96). Auxin also crosstalks with brassinosteroids (BR). We previously (section 2.4)

mentioned the role of auxin transport in vascular bundles development in the inflorescence stem. A computational model indicates that combinatorial action of both auxin and BR is required to explain periodic vascular bundle distribution in the shoot inflorescence (57). At the cellular level, computational modelling, genetics and imaging allowed to show that BRs regulate PILS and as a result auxin levels in the nucleus, identifying a mechanism by which the activity of the two hormones can be coordinated to regulate root growth (131).

Non-hormonal signals also feed into auxin signaling during development. We have mentioned in section 2 the fact that mechanical stresses have been proposed to regulate PIN polarities. These mechanical stresses result from the shape of the tissue as well as growth and the changes in shape that can occur during development (For review: (138)). Mechanical stresses, transmitted to neighboring cells and acting on the orientation of microtubules, can explain a flux-based behavior of PIN in both the shoot and root apical meristem (53, 82) and robustness of auxin distribution (29). In the root, priming of LR not only involves auxin signaling as discussed in section 1 but also a developmental clock, the root clock, that rhythmically specifies lateral root founder cells (92)(For review: (144)). This clock is regulated by auxin and in turn regulates auxin responses that are required to prime LR. Genetics and computational modeling suggest that the ARF7-Aux/IAA18 module regulating auxin signaling during priming is also involved in entrainment of the root clock by external periodic auxin input (108). Another model suggests that periodic changes in cell size of the vasculature could be at the origin of a periodic auxin input (137). However, the same study also proposes a reflux-and-growth mechanism that could rhythmically generate lateral roots without a developmental clock, which in addition to potential oscillatory properties of auxin signaling activity could provide a way for auxin to regulate periodic LR priming at the root tip. Thus, how the root clock and auxin interact remains to be clarified (For further discussion: (107))

but these studies provide an exciting framework to think about how auxin can interact with a complex endogenous developmental program.

3.2 Integration of environmental stimuli with auxin

As highlighted before, auxin existence was first predicted from analyzing the response to environmental stimuli and auxin is a central mediator of growth and developmental responses to such stimuli, the tropic responses. Certain tropisms can be explained by an asymmetric distribution of auxin that leads to asymmetric growth and bending of the organ (Cholodny-Went theory). Auxin transport is therefore fundamental for driving these fast responses and adapting to environmental conditions. Computational modeling has allowed providing essential information on the dynamics of redistribution during one of the most studied tropism, root gravitropism. By modeling the degradation of DII-VENUS induced by auxin signaling (20), Band *et al.* (7) could show that a massive redistribution of auxin occurs within minutes of a gravitropic stimuli, a vision further supported by recent analysis with the AuxSen FRET biosensor (54). Incorporating statoliths in a model also suggests that statoliths sedimentation and PIN intracellular dynamics act at different time scale and can both contribute to the timing of the gravitropic response (75). Combining live-imaging and modeling, Retzer *et al*. (117) further found that BR-mediated formation of a lateral PIN2 gradient is essential to control the timing of root curvature during gravitropism. Still related to the timing of the gravitropic response, modeling together with a synthetic biology approach allowed to demonstrate a contribution of non-transcriptional responses to the regulation of growth in the root, allowing for a very short delay between auxin changes and growth responses (38). Computational modelling has also allowed to explore the fundamental question of how plants maintain the root functional organization during a gravitropic response (81). Modelling, genetics

and imaging predict that the fast root responses to gravity is compatible with maintaining root zonation through the action of the PLETHORA (PLT) transcription factors. Both auxin and PLTs are essential to define root zonation of the root but PLTs are induced by auxin on a longer timescale than a gravitropic response. This separation of the timescales provides a way to maintain stable PLT-mediated developmental zonation during a gravitropic response (81). Modeling has further allowed to implicate H^+-ATPase during the early steps of phototropism (55) and a sequential effect on PIN2, AUX1 and PIN1 during halotropism (136). Together these studies highlight the crucial role of the timing of the many processes involved in the control of a tropic response in order to respond to an environmental stimulus without altering normal plant development. This work also lays the ground for the development of a quantitative, global vision of how auxin distribution can modulate plant architecture in response to environmental conditions and call for the development of an integrative model including also development response to other external signals such as nitrogen that also rely on modulation of auxin transport in the root (105).

Summary points

1. Bioinformatics and computational modelling have become precious tools alongside wet-experiments to understand auxin biology.
2. Omics data from multiple plant species have revealed that auxin transport mechanisms evolved before the auxin signaling machinery, which is a land plants innovation.
3. Whole-genome ARF-binding analysis techniques have revealed different binding properties for ARFs from clade A and B and conservation within a clade.

4. Computational models predict that interactions between the different components of the NAP contribute to different properties of the transcriptional response, allowing to understand the diversification of the NAP components during evolution.
5. Computational models suggest that auxin compartmentalization inside the cell, notably through ER-located auxin carriers, is fundamental for regulating auxin transcriptional responses.
6. At the tissue scale, computational models of auxin transport show that the different efflux and influx carriers, movement through plasmodesmata, as well as biosynthesis and degradation of auxin synergistically regulate auxin gradients during development
7. Computational models have been essential to decipher how internal and external signals act on auxin distribution and vice versa for the coordinated control of slow and fast developmental responses.

Future issues

1. Do class C ARFs target the same genes as class A and B ARFs? Do they play a role in auxin signaling?
2. What are the sequences that are targeted by ARFs *in vivo*? Is the fact that ARFs from a given clade have similar binding properties true *in vivo*?
3. How much does the non-genomic auxin responses interact with the NAP? Can we model this interaction?
4. Can we use synthetic biology for the analysis of polar auxin transport?
5. Are there mechanisms allowing cells to sense auxin fluxes within a membrane or auxin concentration in a neighboring cell? Are cells inherently polarized?

6. Is the interplay between auxin carriers, auxin metabolism and auxin movement through plasmodesmata required to control auxin distribution in all tissues during development? Can we model the dynamics of auxin distribution from all these mechanisms? Can we integrate other internal and external signals?
7. Can we quantify plant development and measure parameters at a scale sufficient to test the existing models of auxin signaling and auxin transport?

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Terms and definitions

Nuclear Auxin Pathway (NAP)

Pathway comprised of TIR1/AFB, Aux/IAA and ARF, and allowing auxin to regulate transcription

Single cell RNA seq (scRNA-seq)

Sequencing approach that allows obtaining the transcriptome of individual cells in a tissue previously disintegrated

Synthetic Biology

Reconstruction of a molecular process from biological components to customize its functionality

Bi-stable system

System with two steady-states where typically noise or a signal can trigger a switch from state 1 to state 2

Sidebars.

1. Sequencing techniques applied to the study of ARFs binding to the genome.

Multiple sequencing techniques allow studying TF-DNA binding (reviewed in (68)). Here we refer to the ones applied to ARF genomic studies.

DAP-seq studies *in vitro* TF binding to the naked genome in three steps. First, extraction and fragmentation of the genome of an organism. Second, *in vitro* or recombinant production of the TF of interest. Finally, *in vitro* formation of a TF-genome fragments complex that is purified permitting isolation and sequencing of the regions bound by the TF (104).

ChIP-seq permits to isolate regions bound by a TF *in vivo*. A first crosslinking step fixates TF-DNA complexes *in vivo*, that are later, extracted and purified permitting to obtain TF target regions. ChIP-seq results are influenced by the chromatin landscape or other TFs or co-factors.

Several sequencing techniques permit to obtain a map of chromatin landscape. The recently developed Assay for Transposase-Accessible Chromatin Sequencing (ATAC-seq) constitutes a faster and more sensitive tool (21). ATAC-seq uses a hyperactive transposase that inserts DNA fragments flanked by adapters into chromatin-accessible regions. ATAC-seq data crossed to ARF DAP-seq data revealed that ARFs are mainly bound to open-chromatin regions.

2. Computational models to simulate auxin signaling and transport

Many of the computational models we discuss use Ordinary Differential Equations (ODEs), where the rate of change of system's state (e.g. a mRNA, a protein), represented as a variable *x*, over time is expressed as a function of *x* and other biological elements and parameters (p) describing the interaction between the elements considered in the model. ODEs allow to quantitatively describing the evolution of the biological system over time and are thus particularly adapted to analyze auxin signaling and transport during development. Middleton et al. (89) and most subsequent auxin signaling models use ODEs with a model structure describing TIR1/AFB-Aux/IAA co-receptor complexes, ARF-Aux/IAA dimers, ARF-ARF dimers, the different monomers and the regulation of the expression of *Aux/IAA* that generate a negative feedback loop. Here, the *Aux/IAA* expression over time is also representative of the dynamics of an auxin target gene. Similarly, PIN-mediated

transport of auxin can be modeled using ODEs that simulate the allocation/deallocation of PIN to a given membrane, using a different function depending on whether a flux-based or concentration-based hypothesis is used, the allocation of any other protein regulating auxin transport, the biosynthesis and turn-over of PIN, other proteins and of auxin, as well as auxin diffusion.

3- Why measuring parameters is essential for constructing and testing models.

Computational models abstract a biological process into a simplified mathematical representation and are powerful tools to test *in silico* the coherence of a set of experimental data and generate new predictions that can be further tested. However, models require to include parameters that, concerning auxin signaling and transport, would typically describe the kinetic constant of interaction e.g. between Aux/IAA and ARF or the rapidity of allocation of PIN to a given membrane. In most cases, these biological parameters are unknown and parameter ranges are explored when testing the model to evaluate whether a given behavior is robust or whether it is only observed in a small portion of the parameter space. When exploring the parameter space, different behaviors of the modelled system can be observed and only one (or a subset of them) can be relevant biologically. Knowledge on biological parameters then allows one to restrict the parameter space and to identify the properties of the model that are biologically relevant. It can also help to discriminate different models (e.g. the flux-based or concentration-based model), if one of the models does not reproduce the biological process when the measured biological parameters are used.

Figures Captions

Figure 1. Evolution of auxin signaling decrypted using omics approaches. Right panels show the existence and number of NAP components in plants and green algae clades. Stars indicate

duplication events (99). Left panels represent evolution of the auxin signaling pathway through co-option of an ARF-based transcriptional regulatory mechanism existent in charophytes leading to the auxin-dependent de-repression mechanism with the acquisition of the TIR1/AFB-Aux/IAA co-receptor complex.

Figure 2. Lessons on the diversity of NAP mechanisms learned from bioinformatics and computational models. a. ARFs bind as dimers three possible double AuxRE configurations depending on the orientation of the two sites (Inverted Repeats, IR; orange; Everted Repeats, ER, yellow; and Direct Repeats, DR, light green). Spacing between both sites also influences binding. Enriched spacings are indicated as the number of nucleotides between two sites in the image. Clade A (dark green) binds all three motifs whereas clade B (red) binds preferentially IR motifs (43, 104, 129). b. An ARF-Aux/IAA interactome identifies three clusters of interaction modules (width of the arrow represents probability of interaction) (139). Each module controls a different property of the auxin response (37). Sensitivity to auxin controlled by ARF-ARF module allows to explain the different zones of sensitivity observed in the SAM (139) c. Reconstruction of the NAP in yeast combined with mathematical models predicts that Aux/IAA degradation times (t) will affect the dynamic of the auxin response (111). d. Mathematical models of Aux/IAA co-expression predict a competition mechanism between Aux/IAAs (19) that could explain the sequential role of different Aux/IAAs in LR formation (111). e. Combination of two mathematical models can explain LR formation. First, a negative feedback loop is predicted to generate an oscillatory response (89) that fits the oscillatory generation of LR in response to auxin along the root. Second, maintenance of auxin response in absence of the signal (memory pattern) can be explained by a combination of negative and positive feedback loops (72).

Figure 3. Intra and intercellular auxin transport. Passive and active transport are represented as discontinuous or continuous arrows respectively. Passive transport happens across the plasma membrane (1), the symplasm (through plasmodesmata, 2) or the apoplast (3). Clusters of polarized PIN proteins mediate auxin efflux (4). ABCB transporters are both influx and efflux active carriers (5). AUX/LAX transporters mediate auxin influx (6). PILS and certain PIN members mediate intracellular transport.

Figure 4. Computational models for auxin transport predict auxin fluxes and concentrations at the cell and organ level. a. Computational models predict that the ER helps controlling auxin concentration in the nucleus through auxin ER transporters (88, 141). b. Two models explain PIN polarization in the membrane. The canalization model (upper part) correctly predicts auxin fluxes and maxima (black spots) in vein formation (10), embryo polarization (142) or phyllotaxis (130). The concentration-based model (bottom part) explains auxin patterns in ovule formation (145) and leaf margin shape (2). PIN proteins are represented as red stripes in the plasma membrane. Predicted auxin fluxes and maxima are represented as black arrows and spots respectively. c. Computational models combining PIN and ABCB, AUX/LAX or plasmodesmata auxin transport improve predictions of auxin distribution and fluxes in the root (6, 48, 85, 86, 93).

Figures

Figure 1

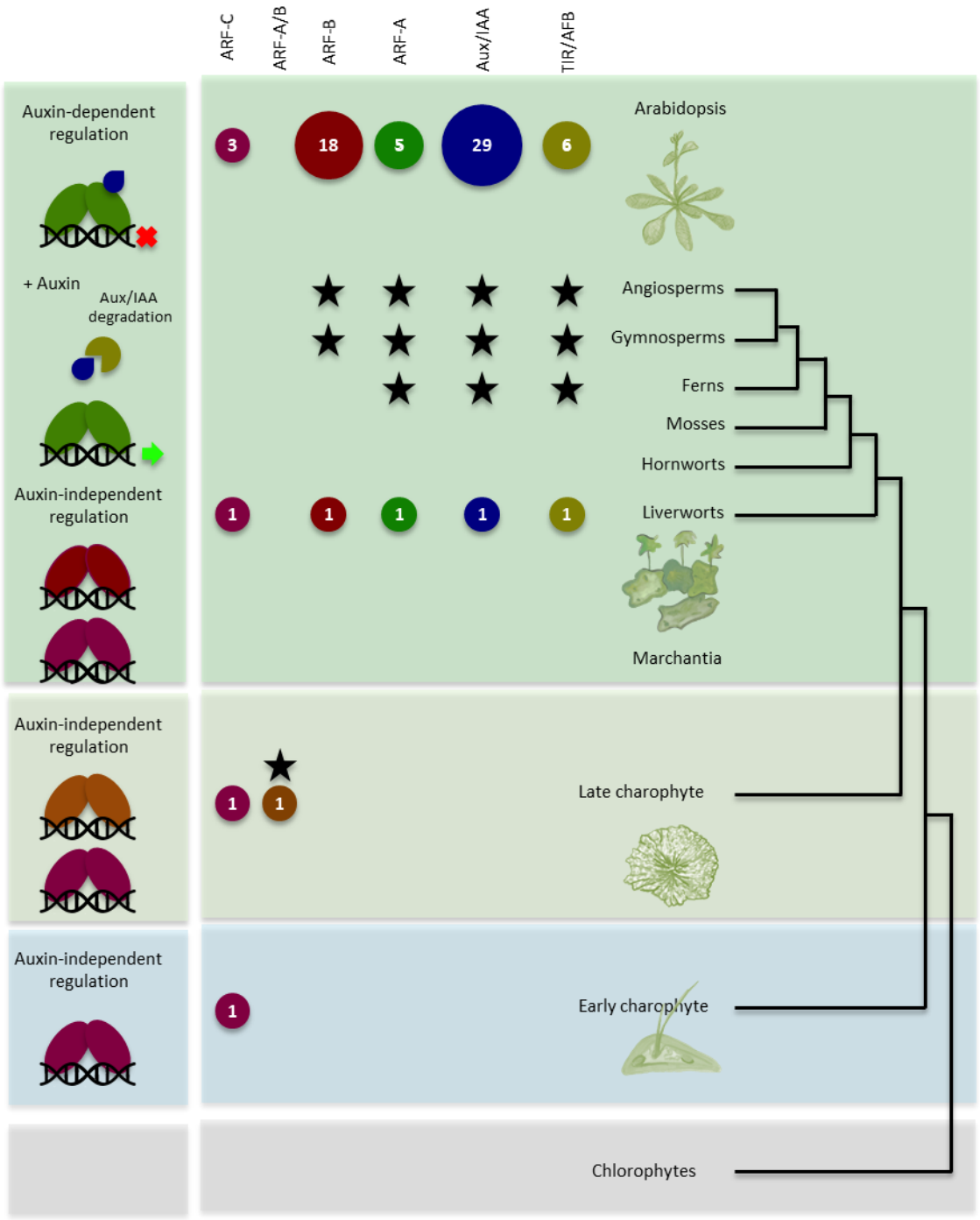
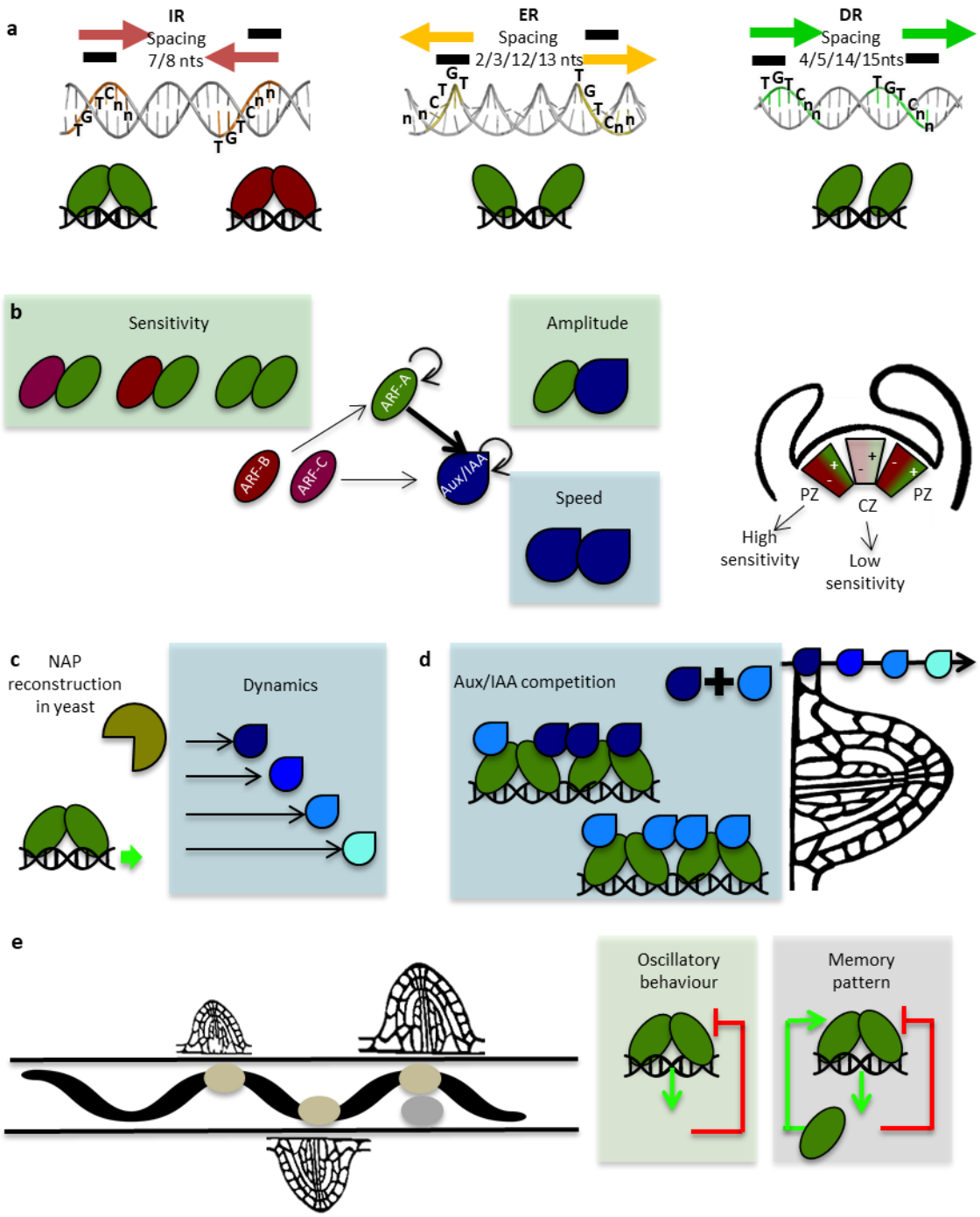


Figure 2


a
IR
Spacing
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ER
Spacing
2/3/12/13 nts
DR
Spacing
4/5/14/15nts
b
Sensitivity
Amplitude
ARF-A
ARF-B
ARF-C
Aux/IAA
Speed
PZ
CZ
PZ
High sensitivity
Low sensitivity
c
NAP reconstruction in yeast
Dynamics
d
Aux/IAA competition
e
Oscillatory behaviour
Memory pattern

Figure 3

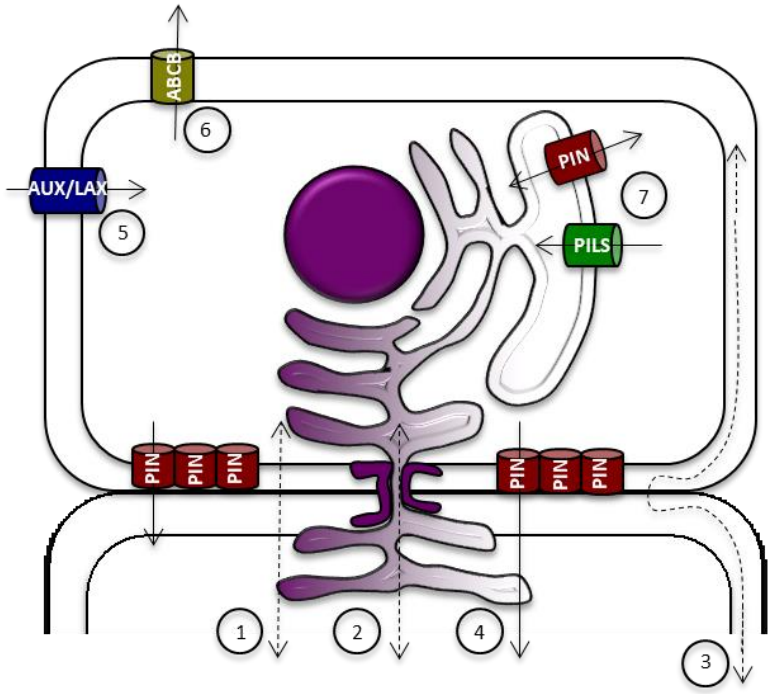
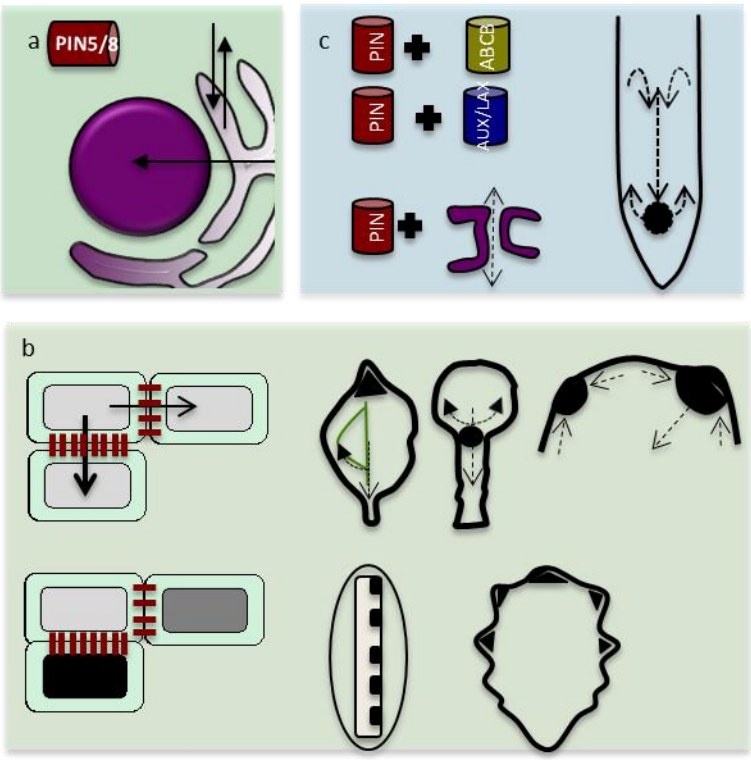
Figure 4