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Mechanisms coordinating auxin metabolism and transport
Mechanizmy koordinácie metabolizmu a transportu auxínu

Bachelor´s thesis

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Dedication

I want to thank my supervisor RNDr. Jan Petrášek, Ph.D., for patience, support, and kindness during the process of writing this thesis.

Declaration:

I declare that I have prepared the final thesis independently and that I have listed all used information sources and literature. Neither this work nor a substantial part of it was submitted for acquisition another or the same academic degree.

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Author's signature

Abstract

Auxin is a small molecule that functions as a plant hormone, and it exists in several forms, of which indole-3-acetic acid (IAA) is the most studied one. IAA modulates cell elongation, division, and differentiation by generating local gradients, and it is essential for almost every aspect of plant growth and development. These gradients are established by the cooperation of IAA biosynthesis, metabolism, and transport. A plant responds to both local auxin maxima and minima; thus, it is necessary to regulate auxin metabolism and transport tightly. However, lots of studies show the roles and regulation of auxin metabolism and transport separately, providing quite rarely discussion on the cooperation of these two processes. Hence, this thesis aims to sum up and refer to mechanisms and regulation of auxin metabolism and transport as a whole, rather than separately, and underline the importance of the cooperation of both auxin metabolism and transport in the plant development.

Key words: auxin metabolism, auxin transport, auxin regulation, auxin biosynthesis, auxin metabolism and transport cooperation, indole-3-acetic acid (IAA), plant development

Abstrakt

Auxín je malá molekula, ktorá funguje ako rastlinný hormón a nachádza sa v rôznych formách, z ktorých je kyselina indol-3-octová (IAA) najštudovanejšia. IAA moduluje rast, delenie a diferenciáciu bunky generovaním lokálnych gradientov a je taktiež dôležitá skoro v každom aspekte rastu a vývoja rastlín. Tieto gradienty sú ustanovené pomocou kooperácie biosyntézy IAA, jej metabolizmu a transportu. Rastlina reaguje na obe auxínové lokálne minima a maxima, a teda je dôležité úzko regulovať metabolizmus a transport auxínu. Veľa štúdií sa zaoberá jednotlivými úlohami a reguláciou metabolizmu a transportu auxínu oddelene, avšak zriedka sa diskutuje o spolupráci a spoločnej regulácii týchto dvoch procesov. Preto je cieľom tejto práce zhrnúť mechanizmy a reguláciu metabolizmu a transportu auxínu, ako celok, nie samostatne, a zdôrazniť význam spolupráce metabolizmu a transportu auxínu v procesoch vývoja rastlín.

Kľúčové slová: metabolizmus auxínu, transport auxínu, regulácia auxínu, biosyntéza auxínu, spolupráca metabolizmu a transportu auxínu, kyselina indol-3-octová (IAA), vývoj rastlín

Contents

List of abbreviations

1-NAA	1-naphthaleneacetic acid
2,4,-D	2,4-dichlorophenoxyacetic acid
4-Cl-IAA	4-chloroindole-3-acetic acid
ABCB	fertilization-independent seed
AFBs	auxin-signaling F-box proteins
AGC	serine/threonine kinases with homology to mammalian protein kinase A, cGMP-dependent kinase, and protein kinase C
AHL	at-hook-containing nuclear-localized protein
AP2	alpheta2
ARF	auxin response factor
ARF GEF	guanosine exchange factor for adenosine-ribosylation-factor-type small GTPases
ASA1	anthranilate synthase alfa subunit 1
ASB1	anthranilate synthase beta subunit 1
Aux/IAA	auxin/indole-3-acetic acid
AUX/LAX	auxin-resistant/like aux
AXR1	auxin resistant 1
BFA	brefelidin
BRM	brahma
CME	clathrin-mediated endocytosis
COI1	coronatine insensitive 1
CRKs	Ca2+/calmodulin-dependent protein kinase-related kinases
CYP71A13	indoleacetaldoxime dehydratase 71A13
CYP79B	cytochrome P450 monooxygenase 79B
D6PK	D6 protein kinase
DAO	DIOXY genes for auxin oxidation
EE	early endosome
EMF	embryonic flower
EML	EMSY-like Tudor/Agent H3K36me3 histone readers EMSY-Like protein
ER	endoplasmic reticulum
ERF	ethylene response factor
ESM1	epithiospecifier modifier 1

ESP	epithiospecifier
ETT	ARF3/ETTIN
FIS	fertilization-independent seed
GA	Gibberellic acid
GH3	Gretchen Hagen3
CHR	chromatin remodeling
IAA	indole-3-acetic acid
IAA-glc	IAA-glucose
IAAId	indole-3-acetaldehyde
IAM	indole-3-acetamide
IAMT1	IAA carboxyl methyltransferase1
IAN	indole-3-acetonitrile
IAOx	indole-3-acetaldoxime
IAR3	IAA-alanine resistant3
IBA	indole-3-butyric acid
IDD	indeterminate-domain
IGs	indole glucosinolates
ILLs	ILR1-like proteins
ILR1	IAA-leucine resistant1
IND	indole
IPyA	indole-3-pyruvic acid
KMBA	α-keto-γ-methiolbutyric acid
LHP1	like heterochromatin 1
LRR	leucine-rich repeat
MAP	mitogen-activated protein
me	methyl
Met	methionine
MPKs	MAP kinases
MRG2	H3K4me3/H3K36me3-binding protein Morf-Related Gene 2
MSI1	multicopy supressor of ira 1
NITs	nitrilase
oxIAA	2-oxindole-3-acetic acid
oxIAA-glc	2-oxindole-3-acetic acid glucose
PAA	phenylacetic acid

PAT	polar auxin transport
PID	pinoid
PIF	phytochrome-interacting factor
PIN	pin-formed
PLT	plethora
PM	plasma membrane
PP2A	protein phosphatase 2A
PRC2	polycomb repressive complex 2
PRS	pressed flower
RLK	receptor-like kinase
S	serine
SPT	bHLH transcription factor SPATULA
SUR	S-alkyl-thiohydroximate lyase
T	threonine
TAA1	tryptophane aminotransferase of arabidopsis 1
TAR	tryptophan aminotransferases
TDCs	tryptophan decarboxylases
TGG	myrosinase
TIR	transport inhibitor response
TMK	transmembrane kinase
TNG	trans Golgi network
TPL	topless
TRA	tryptamine
Trp	tryptophane
UGT74B1	UDP-glycosyltransferase 74B1
UGT74D1	UDP-glycosyltransferase 74D1
UGTs	UDP-glycosyltransferases
VAS1	reversal of SAV3 phenotype 1
VRN	vernalization
WAG	wavy root growth
WOX	wuschel related homeobox
WRI1	wrinkled 1
YUCs	YUCCAs

Introduction

Like all humans, animals, and other multicellular living species, plants also need signalling molecules to function, thrive, and reproduce. For plants, these molecules are called phytohormones. The first-ever known and most studied phytohormone is auxin. It is present in every aspect of plant development and growth. The name auxin, derived from the Greek word αυξειν (auxein), means to increase, to grow (Kögl & Haagen-Smit, 1931). As the name suggests, auxin is essential for plant development, generally through mechanisms that affect cell elongation, division, and differentiation. These are complex mechanisms that require coordination of auxin metabolism, transport, and signalling. Whereas these mechanisms are still not completely understood, and plenty is about to be discovered in the field of auxin biology.

Several molecules are recognized as auxins, and they can be divided into endogenous (natural) and synthetic forms. Endogenous auxins are represented by the most known and studied indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), phenylacetic acid (PAA), and 4-chloroindole-3-acetic acid (4-Cl-IAA). The synthetic auxins, used mainly in commercial applications, are 1-naphthaleneacetic acid (1-NAA) and 2,4-dichlorophenoxyacetic acid (2,4,-D), which is a well-known and utilized herbicide against weed. These auxin forms, both natural and synthetic, differ in their metabolic stability, effective concentration, and transport properties. Although all these forms are unique and worthy of further study, this thesis will only cover the IAA form of auxin. It appears in all plants, and nearly every plant tissue has the capacity for its biosynthesis.

The concentration of IAA in plants is essential due to plants' response, which is threshold-dependent. The low auxin levels typically promote growth, while high auxin levels inhibit growth (Collett et al., 2000). Therefore, the IAA levels must be regulated during plant growth and developmental processes. This thesis aims to sum up all the latest findings of mechanisms that regulate and coordinate auxin metabolism and transport, as it is crucial for understanding the complexity of plants' growth and developmental processes.

1. Mechanisms of auxin metabolism and transport

1.1. IAA metabolism

1.1.1. IAA biosynthesis

There are two auxin biosynthesis pathways described in plants. One is the tryptophan (Trp)-dependent, and the other is the Trp-independent. Both these pathways take place in the cytosol. IAA can also be converted from IBA through an enzymatic reaction, which is reversible and occurs in the peroxisome via β-oxidation (Figure 1) (Zolman et al., 2008). Conversion of IBA to IAA is pertinent for plant development (Strader et al., 2010).

The Trp-independent pathway was proposed after finding that mutants deficient in Trp biosynthesis could produce IAA (Normanly et al., 1993). This pathway converts indole (IND) from chloroplasts to IAA (Figure 1). Although this pathway was detected in many plant species, it is still little known about the intermediates, and it is uncertain which genes are involved.

The most studied IAA biosynthesis pathway is Trp-dependent. Aromatic amino acid L-Trp is synthesized in the chloroplast by the shikimate pathway. The Shikimate pathway produces most of the aromatic amino acids in plants (Maeda & Dudareva, 2012). Several Trp-dependent pathways were proposed; nevertheless, only one Trp-dependent path is wholly described. In this pathway, IAA is produced from L-Trp through indole-3-pyruvic acid (IPyA) in two steps. The first step is a conversion of L-Trp to IPyA. In *Arabidopsis thaliana*, a family of tryptophane AMINOTRANSFERASE OF ARABIDOPSIS1 (TAA1) and TAA1-related proteins catalyse this conversion (Stepanova et al., 2008; Tao et al., 2008). Also, this step is reversible in the presence OF REVERSAL OF SAV3 PHENOTYPE 1 (VAS1) and methionine (Met), which is a source of an amino group. During this reverse reaction, Met is converted to α-keto-γ-methiolbutyric acid (KMBA) (Zheng et al., 2013). The second step is a final conversion from IPyA to IAA by flavin monooxygenase YUCCA family (Zhao et al., 2001). The other, not so well-known pathways take place in plants via tryptamine (TRA), indole-3-acetamide (IAM), and Indole-3-acetaldoxime (IAOx) (Figure 1) (Bak et al., 2001; Douglas Grubb et al., 2004; Gao et al., 2020; Ljung, 2013; Sugawara et al., 2009; Zhao et al., 2001, 2002).

1.1.2. IAA conjugation

The most important are ester-linked IAA conjugates, amide-linked IAA conjugates, and methyl (me) IAA conjugates.

The main ester-linked IAA conjugates are IAA-glucose (IAA-glc) and its derivate IAA-myo-inositol (Jakubowska & Kowalczyk, 2005; Kai et al., 2007). In plants, a group of UDP-

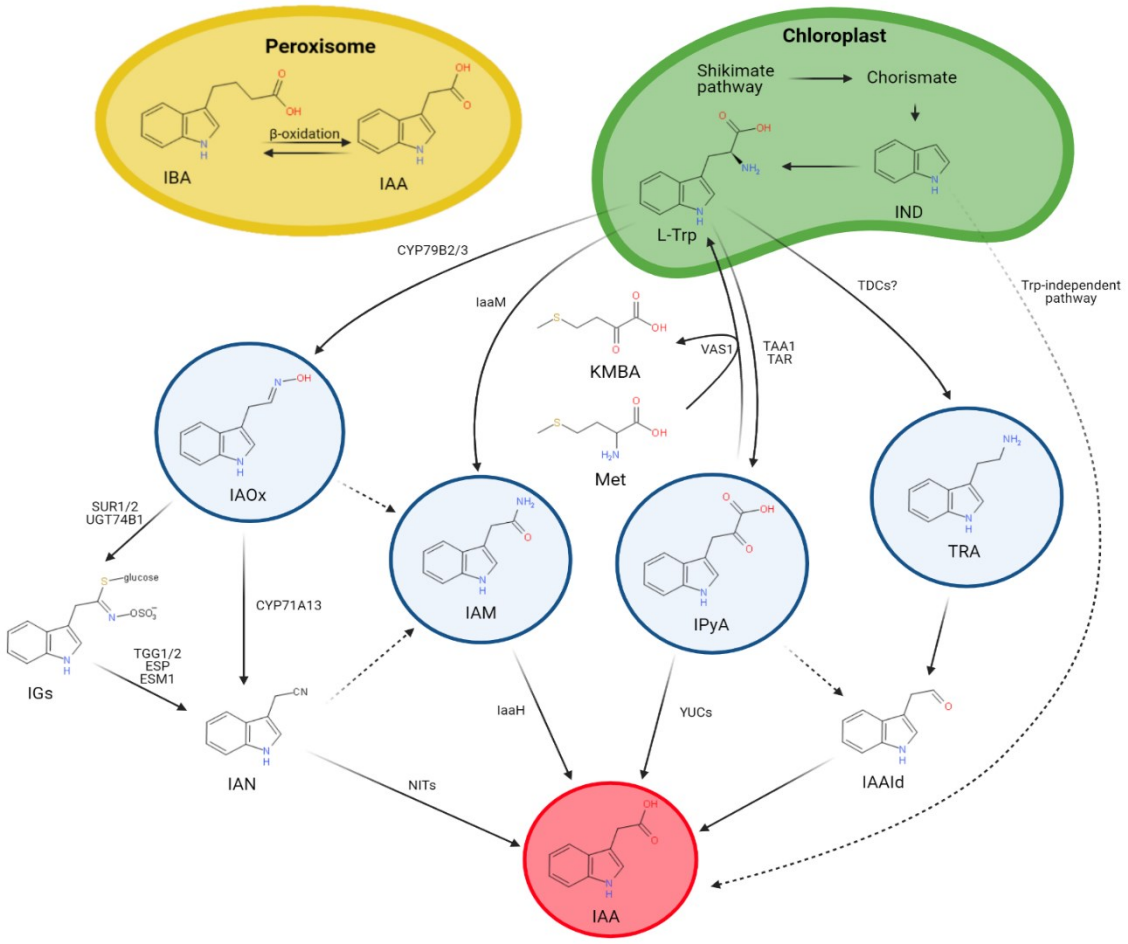

Figure 1. Indole-3-acetic acid (IAA) biosynthesis pathways. Dashed arrows represent pathways, which are unknown or not well-known. Solid arrows represent pathways, which genes, intermediates, or enzymes are studied. IAA is marked with a red circle. The biosynthesis of L-Tryptophan (Trp) takes place in the chloroplast (green bean shape) via the Shikimate pathway (Maeda & Dudareva, 2012). Indole (IND) is produced via this pathway too. The Trp-independent pathway converts indole to indole-3-acetic acid (IAA) (Normanly et al., 1993). This pathway is not well known. The Trp-dependent pathway has multiple ways, and the primary intermediates are marked with a blue circle. The pathway via indole-3-pyruvate (IPyA) is the only complete one (Novák et al., 2012; Stepanova et al., 2008; Tao et al., 2008). IPyA can be converted back to Trp by VAS1 (Zheng et al., 2013). The other pathways are via tryptamine (TRA) (Ljung, 2013; Y. Zhao et al., 2001), indole-3-acetamide (IAM) (Gao et al., 2020), and Indole-3-acetaldoxime (IAOx) (Bak et al., 2001; Douglas Grubb et al., 2004; Sugawara et al., 2009). Indole-3-butyric acid (IBA) conversion via β-oxidation to IAA takes place in the peroxisome (yellow oval) (Strader et al., 2010). The genes forming enzymes, which catalyze the reactions, are given on arrows with smaller font size. CYP71A13 – indoleacetaldoxime dehydratase 71A13; CYP79B – cytochrome P450 monooxygenase 79B; ESM1 – epithiospecifier modifier 1; ESP – epithiospecifier; IAAId – indole-3-acetaldehyde; IGs – indole glucosinolates; KMBA – α-keto-γ-methiolbutyric acid; Met – methionine; NITs – nitrilase; SUR – S-alkyl-thiohydroximate lyase; TAA1 – tryptophane aminotransferase of arabidopsis 1; TAR – tryptophan aminotransferases; TDCs – tryptophan decarboxylases; TGG – myrosinase; UGT74B1 – UDP-glycosyltransferase 74B1; VAS1 – reversal of SAV3 phenotype 1; YUCs – YUCCAs.

glycosyltransferases (UGTs) produce IAA-glc from a free IAA (Jackson et al., 2001; Q. Liu et al., 2019; Ludwig-Müller et al., 2005). The outcome of overexpression of *UGTs* genes is an

increasement of IAA-glc levels and reduction of amide-like IAA levels (Ludwig-Müller et al., 2005). The amide-linked IAA conjugates are IAA-amino acid, IAA-peptide, and IAA-protein. The attached molecule varies between plant species (Staswick, 2009). The *Gretchen Hagen3* (*GH3*) gene family, IAA acyl acid amido synthases, catalyses the bond between amide and IAA (Ostrowski & Ciarkowska, 2021; Staswick et al., 2005). The release of the free IAA from amide-linked IAA conjugates is accomplished through enzymatic hydrolysis via amidohydrolases IAA-leucine resistant1 (ILR1), ILR1-like proteins (ILLs), and IAA-alanine resistant3 (IAR3) (Bartel & Fink, 1995; Davies et al., 1999; LeClere et al., 2002). The benefits of accumulating IAA conjugates are that these storage forms can be rapidly transformed back to free IAA, allowing a plant to achieve high IAA levels in desired tissue in a relatively short time (Ljung et al., 2001). For instance, as shown in pine seeds, the formation of conjugates happens in the phase of seed ripening, and IAA is released during germination (Sandberg et al., 1987). meIAA is also an inactive storage form of IAA. The methylation of IAA is catalysed by IAA carboxyl methyltransferase1 (IAMT1) (Qin et al., 2005). The opposite reaction, release of free IAA, is provided by methylesterase 17 (MES17) and related enzymes that hydrolyse meIAA (Yang et al., 2008). The overexpression of *IAMT1* leads to a reduction of free IAA levels, which can be observed as an agravitropic root growth (Takubo et al., 2020). Transportation of meIAA, which is non-polar, is both through passive influx and PIN-mediated efflux, which can result in a change of the IAA gradients (Abbas et al., 2018).

1.1.3. IAA catabolism

Not all IAA conjugates can be hydrolysed back to free IAA. These are IAA-aspartic acid and IAA-glutamic acid (Östin et al., 1998). Hence, these conjugations belong to IAA catabolism.

The main pathway of IAA catabolism is via oxidation metabolism. It is a conversion of IAA to 2-oxindole-3-acetic acid glucose (oxIAA-glc) in two steps (Novák et al., 2012; Östin et al., 1998; Porco et al., 2016). The first step is irreversible oxidation of IAA to 2-oxindole-3-acetic acid (oxIAA) by *DIOXY* genes for auxin oxidation (DAO) (Z. Zhao et al., 2013). Importantly, this oxIAA cannot be transported from cell to cell by carriers (Pěnčík et al., 2013; Zhao et al., 2013). The second step is the glycosylation of oxIAA to oxIAA-glc by the UGT74D1 enzyme (Tanaka et al., 2014).

1.1.4. Localization of IAA metabolism

As mentioned above, IAA metabolism primarily takes place in the cytosol, where most reactions occur (Figure 2). This supports the fact that auxin biosynthesis and degradation enzymes

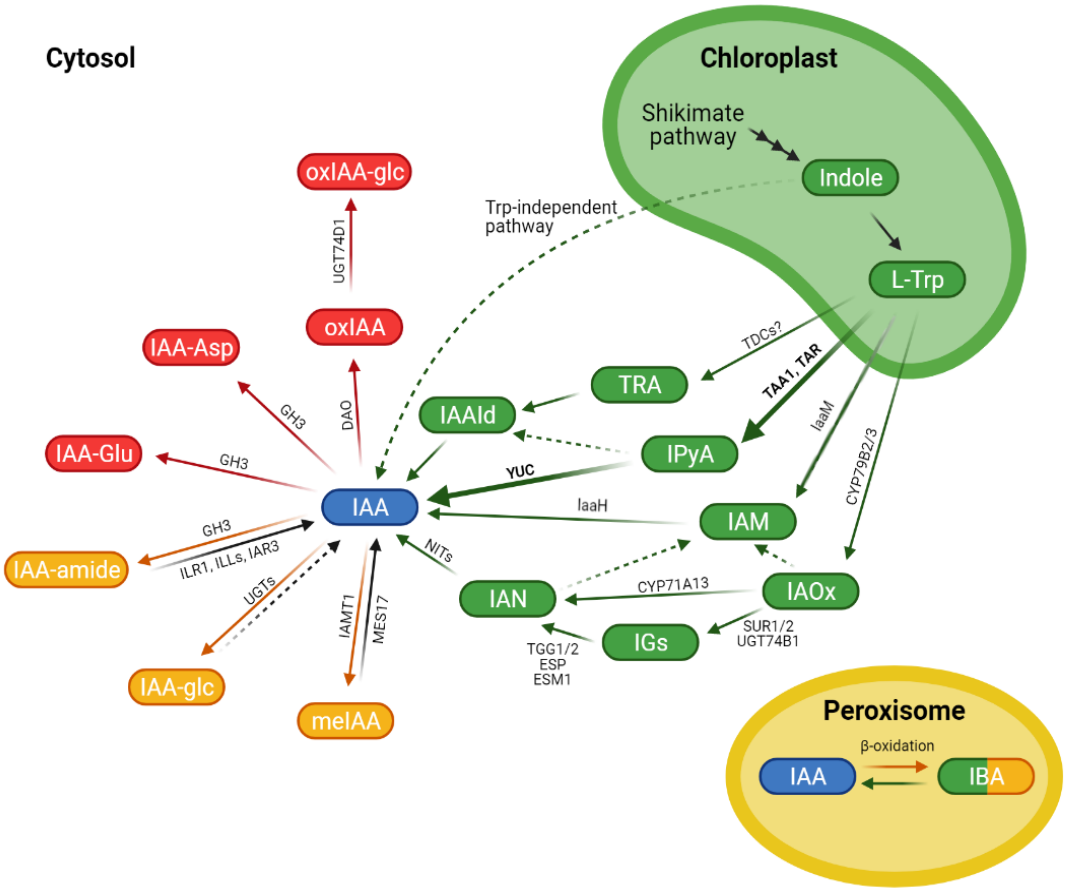

Figure 2. Indole-3-acetic acid (IAA) metabolism. IAA biosynthesis is shown in green (Bak et al., 2001; Douglas Grubb et al., 2004; Gao et al., 2020; Ljung, 2013; Novák et al., 2012; Stepanova et al., 2008; Sugawara et al., 2009; Tao et al., 2008; Y. Zhao et al., 2001, 2002). The shikimate pathway takes place in the chloroplast (green bean shape) (Maeda & Dudareva, 2012). The vice versa conversion of IBA to IAA takes place in the peroxisome (yellow oval) (Strader et al., 2010). Bold arrows mark the main IAA biosynthesis pathway. IAA conversion to storage forms is shown in orange (Davies et al., 1999; Jackson et al., 2001; LeClere et al., 2002; Q. Liu et al., 2019; Ludwig-Müller et al., 2005; Ostrowski & Ciarkowska, 2021; Qin et al., 2005; Staswick et al., 2005; Y. Yang et al., 2008). IAA catabolism is in red (Novák et al., 2012; Östin et al., 1998; Porco et al., 2016; K. Tanaka et al., 2014; Z. Zhao et al., 2013). Dashed arrows represent pathways, which are not well known or unknown. Solid arrows represent pathways, which genes, intermediates, or enzymes are studied. The genes forming enzymes, which catalyse the reactions, are given on arrows in black with smaller font size. CYP71A13 – indoleacetaldoxime dehydratase 71A13; CYP79B – cytochrome P450 monooxygenase 79B; DAO – DIOXY genes for auxin oxidation; ESM1 – epithiospecifier modifier 1; ESP – epithiospecifier; GH3 - Gretchen Hagen3; IAA-glc – IAA-glucose; IAAId – indole-3-acetaldehyde; IAM – indole-3-acetamide; IAMT1 – IAA carboxyl methyltransferase1; IAN – indole-3-acetonitrile; IAOx – indole-3-acetaldoxime; IAR3 – IAA-alanine resistant3; IBA – indole-3-butyric acid; IGs – indole glucosinolates; ILLs – ILR1-like proteins; ILR1 – IAA-leucine resistant1; IPyA – indole-3-pyruvic acid; KMBA – α-keto-γ-methiolbutyric acid; Met – methionine; NITs – nitrilase; oxIAA – 2-oxindole-3-acetic acid; oxIAA-glc – 2-oxindole-3-acetic acid glucose; SUR – S-alkyl-thiohydroximate lyase; TAA1 – tryptophane aminotransferase of arabidopsis 1; TAR – tryptophan aminotransferases; TDCs – tryptophan decarboxylases; TGG – myrosinase; TRA – tryptamine; Trp – tryptophane; UGT74B1 – UDP-glycosyltransferase 74B1; UGT74D1 – UDP-glycosyltransferase 74D1; UGTs – UDP-glycosyltransferases; VAS1 – reversal of SAV3 phenotype 1; YUCs – YUCCAs.

TAA1, YUC1, YUC2, YUC3, YUC6, YUC11, DAO1, and GH3.17 share cytosolic localization (Di

Mambro et al., 2019; Kriechbaumer et al., 2016; Porco et al., 2016; Stepanova et al., 2008; Tao et al., 2008; Zheng et al., 2016). In IAA biosynthesis, the aromatic L-Trp with IND are produced in chloroplasts via the shikimate pathway (Maeda & Dudareva, 2012). Additionally, the vice versa conversion of IBA to IAA occurs in the peroxisome (Figure 1,2) (Zolman et al., 2008). From the enzymatic point of view of IAA biosynthesis, TAR1 is on the plasma membrane, and YUCCA4 is attached to the endoplasmic reticulum (ER) (Mashiguchi et al., 2011; Stepanova et al., 2011).

Both *TAA* and *YUC* gene expressions display tissue-specific localization in roots (Cheng et al., 2006; Stepanova et al., 2008), suggesting that IAA biosynthesis patterns impact plant growth and development. In *Arabidopsis thaliana*, different *YUCCA* genes are expressed in different parts of the plant or even in different development stages. For instance, in the shoot part of a plant, *YUC1* and *YUC4* genes are expressed (Cheng et al., 2006, 2007). Whereas in embryos, *YUC1*, *YUC4*, *YUC10*, and *YUC11* are expressed in apical cells (Cheng et al., 2007). This localization applies to *TAA* and *TAR2* too. *TAA1* is expressed in the quiescent centre, and *TAR2* in the provasculature of meristematic regions (Stepanova et al., 2008).

1.2. Auxin transport

While nearly all plant tissues can produce auxin, most are usually synthesized in young developing plant tissues. Hence, auxin is redistributed across the plant body from its sites of synthesis. Plants can transport auxin via two main pathways. The first is the long-distance and faster transport via the phloem (Morris & Thom, 1978). The second is a polar auxin transport (PAT) (Figure 3), which is slower. It is a cell-to-cell transport through protein carriers. There are four main groups of auxin transporters: AUXIN-RESISTANT/LIKE AUX (AUX1/LAX) auxin influx carriers, PIN-FORMED (PIN) auxin efflux carriers, PIN-LIKES (PILS) transporters, and ATP-BINDING CASSETTE TRANSPORTERS OF THE B CLASS (ABCB). By PAT, auxin is primarily transported in a basipetal direction by cambial cells, although it can also travel in short distances across other tissue types like parenchyma (Galweiler et al., 1998). Because of the tissue-dependent directionality formed by the asymmetric subcellular localization of auxin influx and efflux carrier proteins, the auxin cell-to-cell transport mechanism is unique among plant hormones and signalling molecules. Indeed, the regulated auxin transport has not been detected for any other signalling molecule in the plant species. Auxin can also travel through plasmodesmata (Mellor et al., 2020), enabling the reflux loop between the outer and inner root-tissue layers (Grieneisen et al., 2007).

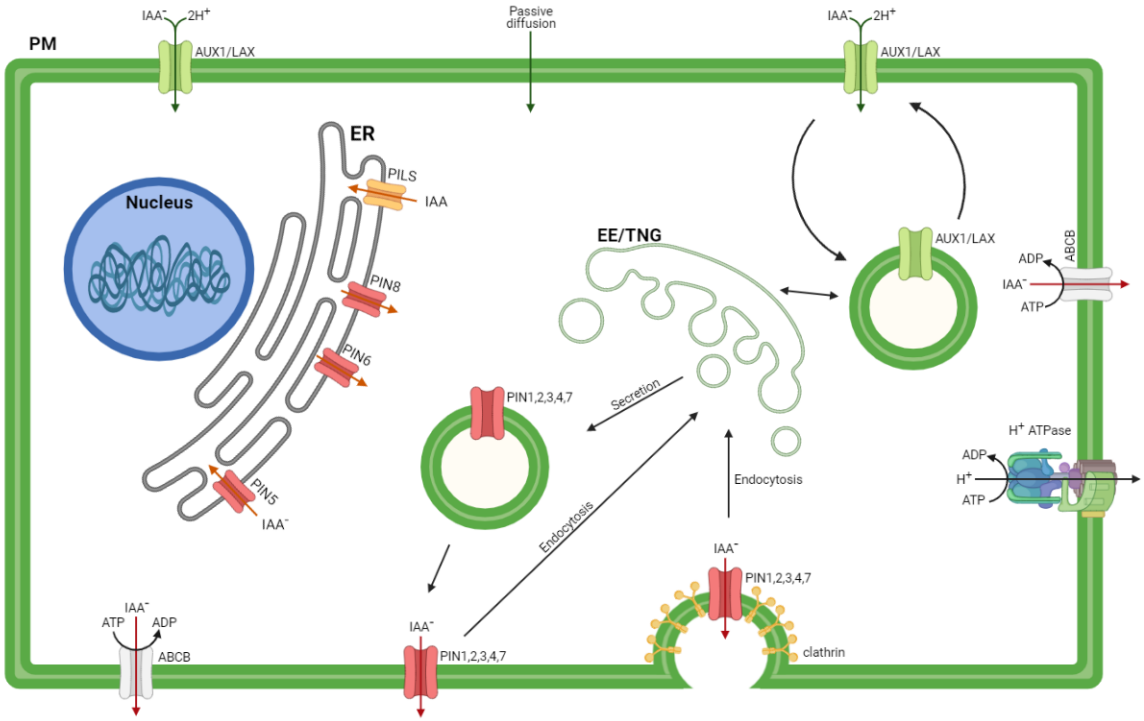

Figure 3. Indole-3-acetic acid (IAA) transport in the cell. Green are AUXIN-RESISTANT 1/LIKE AUX family (AUX1/LAX) auxin influx carriers (Carrier et al., 2008; Jonsson et al., 2017; Lomax et al., 1985). Red are PIN-FORMED carriers (PIN) (Blilou et al., 2005; Dhonukshe et al., 2007; Geldner et al., 2003; Sauer et al., 2006). Orange are PIN-LIKES carriers (PILS) (Feraru et al., 2012). White are carriers from ATP-binding cassette transporters of the B class family (ABCB) (G. Wu et al., 2007). EE - early endosome; ER – endoplasmic reticulum; PM – plasma membrane; TGN- trans Golgi network.

1.2.1. Auxin influx

Molecules of IAA are uptaken by passive diffusion in the undissociated form and by AUX1/LAX protein family transporters (Figure 3) in its dissociated form. AUX1/LAXes are homologs of amino acid permeases (Bennett et al., 1996). Auxin influx carriers contribute to the maintenance of auxin gradients and the maxima needed for canalization to occur throughout the plant (Friml et al., 2003; Reinhardt et al., 2003). The IAA influx via AUX1/LAX proteins is a symport of an anionic IAA with two protons (Figure 3) (Lomax et al., 1985). The AUX1/LAX family in *Arabidopsis thaliana* consists of AUX1 and three homologs, LAX1, LAX2, and LAX3 transmembrane PM proteins (Carrier et al., 2008). AUX1 regulates root gravitropism (Bennett et al., 1996; Ranjan Swarup et al., 2005) by acting upstream of PIN2 (Liu et al., 2018). AUX1 and LAX3 regulate lateral root development (Swarup et al., 2008). LAX2 promotes vascular development (Péret et al., 2012). AUX1, LAX1, and LAX2 work together to control phyllotactic patterning (Bainbridge et al., 2008). Studies have also shown that AUX1 is constantly and dynamically recycled from the PM by recycling endosomes. This recycling uses a pathway

regulated by ECHIDNA, AUXIN RESPONSE FACTOR 1 (ARF1), and BIG proteins (Jonsson et al., 2017).

1.2.2. Auxin efflux

IAA is a weak carboxylic acid, and therefore, it is trapped in its dissociated form in the more acidic intracellular space. Hence, auxin efflux carriers are essential for the export of the IAA molecule from the cell as well as for creating auxin flow and maxima during developmental processes.

PIN auxin efflux carriers are the most studied ones, often having a typical differential distribution on PM (Figure 3) (Blilou et al., 2005). In *Arabidopsis thaliana*, there are eight PINs (PIN1-PIN8). They differ in their expression, localization, and activity, which affects auxin transport. PIN1, PIN2, PIN3, PIN4, and PIN7 are localized on the PM (Figure 3). PIN5, PIN8, and PIN6 are localized on the ER (Figure 3). Although PIN6 is localized on the ER, certain developmental signals can cause the Serine/Threonine site of PIN6 protein to be phosphorylated, resulting in translocation of PIN6 to the PM (Ditengou et al., 2018). The PINs localized on the PM are responsible for auxin efflux and have typically long hydrophobic loop separating several transmembrane domains. The PINs localized on the ER maintain auxin homeostasis in the cell and have a short hydrophobic loop (Křeček et al., 2009). PINs also can alter their subcellular localization, which is a dynamic process allowing the rapid establishment of new polarised auxin paths (Sauer et al., 2006). It has been shown that this is important for plant development, vasculature formation, or regeneration processes (Mazur et al., 2020). This altering is possible because PINs are continuedly cycling from and back to the PM with the help of clathrin-mediated endocytosis (CME) (Figure 3) (Dhonukshe et al., 2007). In *Arabidopsis thaliana*, this cycling of PINs is mediated by GNOM, which is a guanosine exchange factor for adenosine-ribosylation-factor-type small GTPases (ARF GEF). GNOM is also a brefeldin (BFA) sensitive regulator of vesicle budding (Geldner et al., 2003). However, it is still not completely understood how the canalization mechanisms occur and which genes are involved in re-establishing PIN polarity.

The next group of auxin carriers is represented by PILS proteins, which have significant homology with PINs. Although PIN and PILS proteins may appear structurally similar, they evolved independently (Feraru et al., 2012). In *Arabidopsis thaliana*, all the PILS are localized on the ER (Figure 3), and therefore, they contribute to auxin homeostasis in the cell. PILS can modulate differential growth responses by generating auxin minima (Béziat et al., 2017).

Finally, some of the members of the ABCB group of transporters (G. Wu et al., 2007) were shown to act as auxin carriers. There are 29 ABCB proteins described in *Arabidopsis thaliana*, with three pairs performing complementary developmental functions, i.e. ABCB1/19, ABCB4/21, and ABCB6/20 (Jenness et al., 2019). However, other ABCBs might also be involved in auxin transport,

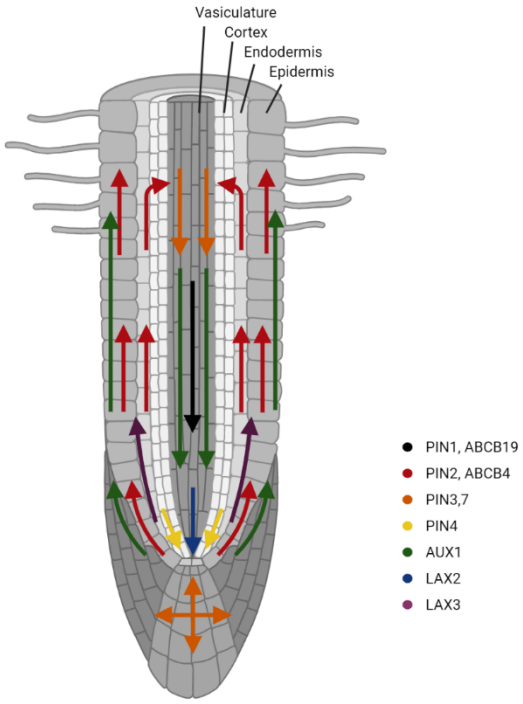

Figure 3. Indole-3-acetic acid (IAA) transport location and direction in the root. The direction of auxin flow in the root is shown with arrows. Each colour of the arrows represent different auxin carriers (Bailly et al., 2006; Michniewicz & Brewer, 2007; Sauer et al., 2006; Swarup, 2001). ABCB ATP-binding cassette transporters of the B class family; AUX1/LAX – auxin-resistant/like AUX1; PIN – PIN FORMED auxin efflux carriers.

as already shown for ABCB14 and ABCB15 being polarly localized in plant tissues (Kaneda et al., 2011). Unlike AUX1/LAX and PIN proteins, whose auxin transport depends on electrochemical gradients, ABCB auxin transport depends on ATP hydrolysis (Figure 3). Several studies have shown that PINs and ABCBs interact and work both individually and interdependently to regulate PAT. Also, ABCB transporters are essential for the equilibrium and proper function of PIN1 carriers (Blakeslee et al., 2007; Mravec et al., 2009; Titapiwatanakun et al., 2009).

1.2.3. Cooperation of auxin carriers

All auxin transporters cooperate within plant tissues, allowing effective redistribution of IAA needed for various developmental events. One of the best-understood example of this co-operation is described for root tip. Here (Figure 4), AUX1 is asymmetrically localized in the root protophloem cells, assisting in phloem unloading and directing the auxin flow in an acropetal (rootward) direction. In contrast, in the lateral root cap and epidermal cells, AUX1 directs the auxin flow in a basipetal (shootward) direction (Swarup, 2001). However, the redirection of auxin flow in the root tip is assisted by auxin flow through PIN proteins, which could be effectively redirected to various PM domains in the root tip tissues (Figure 4). This allows root to react to gravity by differential growth (Kleine-Vehn et al., 2010). Importantly, PIN auxin efflux carriers act already

during the specification of apical-basal axis formation during early embryogenesis, determining the presumptive root pole (Friml et al., 2003).

The primary role of ABCBs is in meristematic tissues with elevated auxin concentrations, where they export IAA into the apoplast (Bailly et al., 2006).

2. Regulation of auxin metabolism and transport

2.1. Metabolic regulation

Various metabolic regulations control auxin metabolism. Conversion of IAA-amide, IBA, and meIAA to free IAA up-regulates the expression of genes associated with the IPyA biosynthesis pathway (Spiess et al., 2014). This indicates that plant cell reacts to low IAA levels not only by the conversion of IAA storage forms or by IAA synthesis *de novo* separately, but rather by co-regulating both processes together. This co-regulation can also be applied for the IAA oxidation, as the oxidative catabolism of IAA up-regulates GH3, as well as IAA biosynthesis *de novo* (Mellor et al., 2016; Porco et al., 2016). As mentioned in chapter 1.1.2., GH3 enzymes catalyse auxin conjugation (Figure 2). With these mechanisms, a plant cell can regulate the auxin homeostasis and react to high or even low auxin levels.

VAS1 can regulate the IPyA-dependent auxin biosynthesis. As mentioned in chapter 1.1.1, VAS1 converts IPyA back to Trp (Figure 1). Recent discovery shows that UGT76F1 can glycosylate IPyA to IPyA-glc, and thus lowering the IPyA concentration available for IAA biosynthesis (Chen et al., 2020). By reversing or transforming the IPyA, plan cells can stop the IAA biosynthesis faster and react faster to rising IAA levels.

PINs localized on the ER modulate the IAA metabolism and thus contribute to the distribution and availability of free IAA in plants. Specifically, in *Arabidopsis thaliana*, overexpression of *PIN5* leads to the elevation of IAA-Asp and IAA-Glu and a decrease of free IAA levels (Mravec et al., 2009). On the other hand, overexpression of *PIN8* results in reduced amounts of IAA-Asp and IAA-Glu and oxIAA. Also, free IAA levels are moderately increased (Ding et al., 2012). However, overexpression of *PIN6* shows higher levels of free IAA as well as IAA-Asp (Simon et al., 2016). Hence, PINs on the PM can modulate the IAA levels inside the cell and thereby modulate the IAA homeostasis.

Both auxin metabolism and transport are regulated by feedback mechanisms dependent on the auxin levels (Ljung et al., 2002; Paciorek et al., 2005; Vieten et al., 2005). Inhibitory feedback mechanisms regulate auxin biosynthesis. These mechanisms work through signalling, which down-regulates the genes involved in the IPyA biosynthesis pathway, resulting in decreased endogenous auxin levels (Suzuki et al., 2015). In contrast, auxin promotes most PINs through auxin/indole-3-acetic acid (Aux/IAA)-dependent signalling. PIN5 is an exception, and auxin actually down-

regulates its expression (Mravec et al., 2009). Next, it has been shown that also LAX3 is regulated by auxin through positive feedback, which increases the auxin flow to the cell. Thus, increasing the auxin levels in the cell (Mellor et al., 2015). ARF proteins mediate this positive feedback and activate *GH3* too. GH3 assembles conjugates that both belong to the storage and catabolism forms of auxin (Figure 2). Hence, GH3 decreases the free auxin levels, but it has been shown that this effect has only impact over a short period of time, and it turns out to be ineffective during more extended periods (Mellor et al., 2016a).

In conclusion, auxin regulates its concentration in the cell by altering the expressions of genes involved in biosynthesis, metabolism, and transport. This altering results in suspending the IPyA biosynthesis pathway, which is the primary pathway of IAA production. Also, in short periods of time, IAA conjugation production is higher, resulting in IAA inactivation and lowered free IAA levels. Then, the auxin flow is promoted as auxin influx LAX3 carriers, and auxin efflux PIN carriers are up-regulated. Furthermore, down-regulation of PIN5 transporter carriers lowers the auxin flow to the nucleus.

2.2. Hormonal cross-talk

Other plant hormones can control auxin metabolism and transport by influencing enzymes involved in auxin metabolism and altering auxin carrier expression, localization, and activity. Hormone cross-talk mechanisms are essential and allow a plat to respond developmentally to environmental stimuli. In this thesis, only cytokinin, ethylene, jasmonate, and gibberellic acid (GA) will be mentioned as cross-talk hormones with auxin. Although, other phytohormones affect auxin metabolism and transport too.

2.2.1. Cytokinin

Cytokinin and auxin collaborate closely not only in regulating cell division and differentiation but also in influencing one another. In fact, they interact on many levels. Although not all direct molecular links for the regulation of auxin by cytokinin are known, several cytokinin effects on auxin metabolism and transport have been proposed.

In *Arabidopsis thaliana*, cytokinin modifies the IAA biosynthesis *de novo*, which is controlled by auxin-cytokinin signal transduction (Jones et al., 2010). Specifically, cytokinin up-regulates *YUC1* and *YUC4* genes in gynoecium primordium and *YUC8* in roots (Di et al., 2016; Müller et al., 2017). In the development processes of the gynoecia medial tissues, cytokinin and the bHLH transcription factor spatula (SPT) co-activate *TAA1* and *PIN3*. Further investigations showed that Arabidopsis response regulator 1 (ARR1), a cytokinin response repressor, mediates *TAA1* activation (Reyes-Olalde et al., 2017). ARR1 also regulates the auxin biosynthesis in the steam cell niche by up-regulating the expression of anthranilate synthase beta subunit 1 (ASB1) (Moubayidin

et al., 2013). ASB1 catalyses tryptophan biosynthesis in the rate-limiting step (Stepanova et al., 2005). Altogether, cytokinin can regulate the whole IPyA biosynthesis pathway. More clear is the cytokinin regulation of auxin degradation, as the ARR1 directly attaches to *GH3.17* activating its transcription and thereby promoting auxin degradation (Di Mambro et al., 2017).

PAT is regulated by cytokinin through transcriptional and post-translational regulations. In roots, cytokinin interacts with the PIN1 carriers, and it promotes retargeting of PM PIN1 proteins to the vesicle, where a lytic degradation occurs (Marhavý et al., 2014). This retargeting reduces the numbers of PIN1 carriers on the PM. BFA-sensitive trafficking pathway regulatory compounds, involving BIG family protein ARF GEF BEN1 and Sec1/Munc18 family protein BEN2, regulate this cytokinin-mediated retargeting (Tanaka et al., 2013). Interestingly, cytokinin-mediated retargeting does not regulate all and only the PIN1 carriers. Lots of PIN carriers tend to be insensitive to the cytokinin, depending on the polar localization. For instance, apical-located PIN1 and PIN2 in epidermal cells were insensitive to cytokinin, whereas basal-located PIN1 in epidermal cells, and basal-located PINs in cortex cells, regardless of the PIN variant, were significantly reduced from the PM by cytokinin treatment (Marhavý et al., 2014). This fine-tuning of the PAT carriers can be crucial for maintaining the apical root meristem or lateral root organogenesis. The basally-localized PIN1 proteins could direct auxin flow carrier towards the newly establishing primordia (Marhavý et al., 2014). Cytokinin can also regulate PIN levels in a shoot by post-translation regulation mechanisms. Unlike the regulation mechanisms in roots, cytokinin actually up-regulates the shoot localized PINs, like PIN3, PIN4, and PIN7. Thus, promoting the shoot brunching (Waldie & Leyser, 2018).

Clearly, cytokinin cross-talk effects regulate metabolism and transport of IAA in the context of developmental processes. However, the individual intermediates and genes involved in these complex mechanisms are unknown, and we can only hypothesize how the auxin and cytokinin co-operate in the developmental processes.

2.2.2. Ethylene

Ethylene is a hydrocarbon gas phytohormone, which is essential for some plant developmental processes, such as fruit ripening, leaf abscission, senescence, or even regulating the responses to biotic and abiotic stress factors (Dubois et al., 2018).

Auxin biosynthesis is increased in response to ethylene. This mechanism was found to take place via ethylene response factor 1 (ERF1), which directly targets and up-regulates *anthranilate synthase alfa subunit 1* (*ASA1*) expression (Mao et al., 2016). ASA1, like ASB1, catalyses tryptophan biosynthesis (Stepanova et al., 2005). Therefore, ethylene up-regulates the IAA biosynthesis, resulting in increased IAA levels, thereby inhibiting root growth.

In the root, ethylene regulates the expression of various PAT components by transcriptional regulation of the carriers' genes. For instance, ethylene-mediated regulation of PAT is essential for apical hook development. The important part of the apical hook development is the curvature caused by asymmetrical cell elongation of the hypocotyl, which is bending (Raz & Koornneef, 2001). By this process, ethylene not only coordinates PAT carriers but also regulates the IAA biosynthesis *de novo* (Zadnikova et al., 2010), as mentioned above via ASA1. Thereby ethylene can regulate the localization of auxin maxima in the hypocotyl. Both AUX1/LAX and PIN carriers are involved in this regulation of the maxima localization by membrane trafficking processes (Figure 3).

2.2.3. Jasmonate

The following important cross-talking phytohormone is jasmonate and its methylester, a phytohormone responsible for growth, photosynthesis, and response regulation to biotic and abiotic stress factors. Coronatine insensitive 1 (COI1) is the receptor for jasmonate, which, when activated, promotes ubiquitination and degradation of jasmonate transcription repressors (Katsir et al., 2008). Thus, promoting jasmonate genes, which then can interact with auxin metabolism and transport.

Like ethylene, methylester jasmonate up-regulates the IAA biosynthesis via ASA1, which is a core of the jasmonate-mediated metabolic cross-talk (Sun et al., 2009). This mechanism depends on ethylene response factor 109 (ERF109), which, due to increased jasmonate levels, also binds to the *YUC2* promoter and stimulates auxin biosynthesis (Cai et al., 2014). *YUC8* and *YUC9* expression are as well promoted by methylester jasmonate, indicating that they could be involved in jasmonate-mediated biosynthesis regulation (Hentrich et al., 2013).

Additionally, methylester jasmonate modulates PIN2 localization and trafficking, but it has not been shown to modulate the PIN2 protein synthesis *de novo*. The jasmonate-modulated auxin transport response depends on the methylester jasmonate concentrations (Sun et al., 2009). These responses conclude COI1 and auxin resistant 1 (AXR1) proteins. It has been shown that by high methylester jasmonate concentrations, PIN2 accumulation on the PM is reduced. By low methylester jasmonate concentrations, PIN2 endocytosis is inhibited (Sun et al., 2011). Interestingly, these effects are most likely dependent on ASA1 and transport inhibitor response 1 (TIR1)/auxin-signaling F-box proteins (AFBs). These suggestions come from findings that by low jasmonate-mediated effects, *asa1* and *tir1/afbs* mutants show reduced PIN2 endocytosis inhibition (Sun et al., 2011). Thus, low jasmonate concentrations are stimulating auxin biosynthesis, which influences the inhibition of PIN2 endocytosis. Whereas by high jasmonate-mediated effects, *asa1* and *tir1/afbs* mutants show enhanced, rather than lowered, reduction of PM PIN2 (Sun et al., 2011). Therefore, auxin biosynthesis probably plays an insignificant role in high jasmonate-modulation effects.

2.2.4. Gibberellic acid

Gibberellic acid and IAA interaction demonstrated on *Arabidopsis thaliana* root exposed to gravitropism stimulation show that GA, like IAA, assembles on the not elongating side of the root (Löfke et al., 2013). It has been shown that high GA levels promote the accumulation of PIN2 on the PM, where es the low GA levels correspond with decreased PIN2 on the PM (Löfke et al., 2013). Therefore, similar to jasmonate, coordination of the PIN2 subcellular trafficking is dependent on the concentration of GA.

Newest findings propose that GA co-modulates IAA biosynthesis and PAT. It has been shown that GA3 promotes local IAA biosynthesis by up-regulating *YUC6* genes in rice, which results in root elongation inhibition (Li et al., 2020). Also, *bHLH102*, *OsMYBR1*, and *OsMyb1R*, rice genes homologous to genes in *Arabidopsis thaliana* included in auxin biosynthesis, were up-regulated by the GA3 treatment (Li et al., 2020). Next, in rice root, GA down-regulates *PIN9* and PILS gene *PIN7b* (Li et al., 2020).

Hence, based on this, it could be hypothesized that gibberellic acid coordinates both auxin metabolism and transport and these mechanisms are essential for the plant development.

2.3. Transcriptional regulation

There is still quite fragmentary information on the transcriptional regulation of auxin metabolism and transport. However, several transcription factors and epigenetic regulators associated with the regulation of auxin during specific developmental responses in plants have been identified.

The molecular switching between active and repressed transcription sites is associated with posttranslational histone modifications. Specifically, it is regulated by different modifications of histone repressive mark H3K2me3, which control auxin genes included in auxin biosynthesis, storage, catabolism, transport, and signalling as well. Namely, auxin biosynthesis genes *YUCs*, *CYPs*, *TAA1/TARs*, *SUR1*, and *NITs*, auxin storage and catabolism genes *GH3* and *IAMT*, auxin transport genes *PINs* and *AUX/LAXs*, and auxin signalling genes *TIR1/AFBs*, *IAAs*, and *ARFs* (He et al., 2012; Lafos et al., 2011). It indicates that understanding the histone modification regulation is essential for understanding the transcriptional regulations during plant development. The significance of H3K2me3 modifications supports the fact that plants have a mechanism of re-establishing reduced H3K2me3 levels, for instance, after DNA replication, histone exchange, or demethylation. In this mechanism subunit, the multicopy suppressor of ira 1 (MSI1) of polycomb repressive complex 2 (PRC2) links PRC2 with helper protein like heterochromatin 1 (LHP1). The PRC2-LHP1 complex then targets chromatin for re-establishing the H3K2me3 levels (Derkacheva et al., 2013). Interestingly, it has been shown that the PRC2-LHP1 recruits and promotes the

expression of auxin biosynthesis *YUC1*, *YUC2*, *YUC4*, *YUC5*, *YUC6*, *YUC8*, and *YUC9* genes (Rizzardi et al., 2011).

DNA methylation and small RNAs can regulate the transcription of genes as well. DNA methylation is associated with the silencing of targeted genes, and it shows to be involved in auxin homeostasis. In plants, DNA methylation happens at CG dinucleotide regions, CHG and CHH trinucleotide regions (C, cytosine, G, guanine, H, one of adenine, thymine, or cytosine). DNA methylation of various genes of auxin metabolism and transport is essential for plant developmental processes and reactions to biotic and abiotic stress factors (Zhang et al., 2018).

During plant developmental processes like flower determination, it is crucial to tune the auxin levels finely. Firstly, *YUC4* genes are up-regulated by chromatin remodelling 11 (CHR11) and CHR17 factors in the phase of flower primordium formation (Yamaguchi et al., 2018). Later on, transcription factor SUPER-MAN (SUP) down-regulates *YUC4* as well as *YUC1* genes (Xu et al., 2018). Thus, reducing the IAA biosynthesis and lowering the IAA levels in the tissue. The inhibition of IAA biosynthesis by SUP is connected with trimethylation of H3K2me3 by PRC2 (Xu et al., 2018). Hence, confirming the importance of transcription regulation by H3K2me3 modifications.

PRC2 also plays an important role in the fertilization process as the fertilization-independent seed (FIS)-PRC2 complex inhibits auxin biosynthesis in the central cell of the ovule (Figueiredo et al., 2015). Specifically, it has been shown that the FIS-PRC2 complex down-regulate *YUC10* genes. Additionally, before fertilization, embryonic flower (EMF)/vernalization (VRN)-PRC2 complex represses the integuments to develop into a seed coat (Figueiredo et al., 2015). Further studies uncovered that also EMSY-like Tudor/Agent H3K36me3 histone readers EMSY-Like protein 1 (EML1) and EML3 repress not only auxin biosynthesis but auxin transport and signalling in the non-fertilized ovule (Milutinovic et al., 2019). Namely, auxin transport carriers *LAX2*, *LAX3*, *ABCB4*, and *ABCB19* genes. Altogether, PRC2, EML1, and EML3 repress auxin biosynthesis, transport, and signalling before fertilization, thereby preventing the non-fertilized ovule from developing into an empty seed.

Lateral organ morphogenesis and gravitropism are co-regulated by indeterminate-domain (IDD) proteins IDD14, IDD15, and IDD16, by promoting transcription levels of auxin biosynthesis and transport genes *YUC5*, *TAA1*, and *PIN1* (Cui et al., 2013). In root developmental processes, *PIN1* and *PIN4* are up-regulated by *Arabidopsis thaliana* MADS-box gene family transcription factor XAANTAL2 (XAL2/AGL14) by directly binding to *PIN* promoter sequences (Garay-Arroyo et al., 2013). This mechanism is selective because, as it has been shown, XAL2 does not regulate *PIN2* or *PIN7* expression (Garay-Arroyo et al., 2013). Additionally, gravitropism is regulated by R2R3-MYB transcription factor four lips (FLP) co-operating with its paralogue MYB88 (H.-Z. Wang et al., 2015). They co-regulate the expression of *PIN3* and *PIN7* genes, which are responsible for the gravitropism responses in the root tip. Further, it has been proposed that in lateral root

formation, expression of *PIN3* is dependent on the direct binding of ARF7 to the *PIN3* promoter region (Chen et al., 2015). ARF7 also controls *FLP* expression (Chen et al., 2015). Therefore, the cooperation of both transcription factors FLP and ARF7 is required for *PIN3* regulation during lateral root development.

Another ARF transcriptional factor ARF3/ETTIN (ETT), has been shown to directly targeting biosynthesis, inactivation, transport, and other signalling auxin genes (Simonini et al., 2017). ETT, which expression is affected by auxin, is suggested to regulate the auxin dynamics itself. ETT was found to both up-regulate and down-regulate the genes connected to auxin metabolism, transport, and signalling. Namely, ETT regulates the expression of auxin biosynthesis *YUC4*, *ASB1*, and *TAA1* genes, auxin storage, and catabolism *ILR1*, *GH3.5*, *GH3.10*, and *IAMT1* genes, both auxin efflux and influx carriers *PIN1*, *PIN3*, *PIN7*, *AUX1*, *LAX1*, and *ABCB19* genes, and signalling genes like *ARF16* and *ARF17* (Simonini et al., 2017). Interestingly, these studies suggest that ETT as well controls genes belonging to other phytohormone pathways, such as ethylene and jasmonic acid (Simonini et al., 2017). Therefore, ETT may serve not only by regulating auxin dynamics but also as a central node for hormonal crosstalk.

In root tip, brahma (BRM), an SWI/SNF ATPase, up-regulates *PIN1*, *PIN2*, *PIN3*, *PIN4*, and *PIN7* genes by targeting and remodelling chromatin in the regulatory elements (Yang et al., 2015). BRM also regulates the *transcription of plethora* (*PLT*) genes *PLT1* and *PLT2*, which are responsible for the maintenance of stem cell niche (Yang et al., 2015). This indicates that BRM acts in the PLT pathway and thereby regulates the maintenance of stem cell niche.

The connection between auxin conjugation and the transport was spotted by studies of an alpheta2 (AP2) transcription factor wrinkled 1 (WRI1) (Kong et al., 2017). This transcription factor regulates fatty acid and oil synthesis in plants (Cernac & Benning, 2004). WRI1 deploys on the *CH3.3* promoter and down-regulates its expression. Interestingly, in *wri1-1* mutants, the IAA-Asp levels were elevated, whereas the free IAA levels were not altered (Kong et al., 2017). WRI1 also up-regulates *PIN1*, *PIN3*, *PIN5*, and *PIN6* genes. Further, it has been shown that WRI1 only binds to *PIN4* and *PIN5* promoters AW-box but does not bind to *PIN1* and *PIN6* promoter regions (Kong et al., 2017). Altogether, although the connection of auxin conjugation and transport via WRI1 is not fully understood, clearly, WRI1 co-regulates the expression of *CH3.3*, *PIN1*, *PIN3*, *PIN5*, and *PIN6*. Besides, GH3 probably functions downstream of PINs with the ER localization (Mravec et al., 2009).

In addition, biotic and abiotic stress factors modulate auxin metabolism via transcription regulation. For instance, in normal light conditions, at-hook-containing nuclear-localized protein 27 (ALH27) and AHL29 repress *YUC9* gene expression by directly binding to the *YUC9* locus (Lee & Seo, 2017). The repression mechanism blocks the RNApol II to access the *YUC9* promoter by depositing the histone H2A.Z. In eukaryotes, the deposition of H2A.Z is promoted by SWI2/SNF2-related 1 (SWR1) complex, which is recruited by AHLs (Lee & Seo, 2017). As a result, when the

hypocotyl grows in the shade, YUC9 is expressed. A similar mechanism occurs with temperature and light regulation of *YUC8*. In response to shadow, phytochrome-interacting factor 7 (PIF7) and H3K4me3/H3K36me3-binding protein Morf-Related Gene 2 (MRG2), recruited by PIF7, bind to the *YUC8* promoter, thereby repressing it (Peng et al., 2018). H2A.Z is removed by histone deacetylase 9 (HDA9) at higher temperatures, allowing the RNApol II to access the YUC8 promoter and thereby activating the *YUC8* transcription (van der Woude et al., 2019).

2.4. Post-translational modifications

Post-translational modifications consist of covalent modifications that overall affect protein folding and function. One of the main post-translation modifications is phosphorylation, which, as it has been shown, is essential for nearly every aspect of cellular activity. Phosphorylation acts like an on/off switch of the protein activity and is catalysed by kinases, which catalyse the phosphorylation reaction, and phosphatases, which reverses phosphorylation.

In plants, the main kinases come from the receptor-like kinase (RLK) family (Mergner et al., 2020). The leucine-rich repeat (LRR)-RLK subfamily functions with transmembrane kinase family (TMK) TMK1, TMK2, TMK3, and TMK4 as crucial regulators of auxin metabolism, transport, and signalling (Wu et al., 2016). TAA1 can be phosphorylated at a conserved threonine 101 (T101) site (Wang et al., 2020). This phosphorylation is mediated by TMK4, which is, in this case, auxin-responsive (Wang et al., 2020). Thus, creating a negative feedback loop by auxin homeostasis and signalling. Further, TAA1 phosphorylation enables phosphorylated TAA1 to dimerize with TAR, thereby regulating the auxin biosynthesis (Wang et al., 2020). YUC proteins can also be phosphorylated. However, the mechanism and kinase responsible for this reaction remind unknown.

The auxin catabolism via DAO activity is barely induced by auxin itself. Nevertheless, DAO can be regulated by post-translation modification via substrate-mediated multimerization, formatting DAO dimers, which is triggered by IAA (Takehara et al., 2020). DAO dimers tend to have raised affinity for IAA.

Tree main protein kinases families AGCIIV kinases (serine/threonine kinases with homology to mammalian protein kinase A, cGMP-dependent kinase, and protein kinase C), Ca2+/calmodulin-dependent protein kinase-related kinases (CRKs), and mitogen-activated protein (MAP) kinases (MPKs) regulate PIN phosphorylation, which is crucial for polar PIN distribution.

Plant-specific AGCVIII kinases can be divided into two subfamilies, which mediate PIN auxin transport. These are D6 protein kinase (D6PK) and pinoid (PID) with its close homologues wavy root growth 1 (WAG1) and WAG2 subfamilies. Both of them are required for polar PIN transport activation (Zourelidou et al., 2014). PID with WAG1 and WAG2 regulate the activity of PIN carriers on the PM by directly phosphorylating the PIN carriers at three conserved serine (S)

sites S1, S2, and S3 (Dhonukshe et al., 2015). It has been shown that PID, WAG1, and WAG2 play a role in regulating directional root growth, phototropism, or apical hook opening (Ding et al., 2011; Haga et al., 2014; Santner & Watson, 2006; Willige et al., 2013). Besides, PIDs and WAGs mediate the PIN3 re-localization to gravitropism stimuli as well (Ding et al., 2011). Furthermore, PIDs are so far the only known kinases in plants able to phosphorylate and inactivate ABCB1 carriers. This inactivation can be reversed by quercetin (Henrichs et al., 2012). Altogether, PIDs kinases activity appears to be an essential auxin efflux modifier and regulator of the polarised auxin flow.

D6PK, like PID, WAG1, and WAG2, phosphorylates PIN proteins at S1, S2, and S3 sites and additionally at S4 and S5 sites. PIN1 lacks S5 and PIN2 lacks both S4 and S5 sites. However, PIN3, PIN4, and PIN7 have both of these additional serine sites (Zourelidou et al., 2014). Differ from PID, WAG1, and WAG2, D6PK does not have an impact on the polarity of PINs and are localized on the basal site of the cell (Zourelidou et al., 2014). D6PK is responsible for phototropism, shade-avoidance, or negative gravitropism (Willige et al., 2013; Zourelidou et al., 2009).

In *Arabidopsis thaliana*, one of the eight CRKs is CRK5, which is known to phosphorylate PIN2 carriers (Rigó et al., 2013). Typically, CRK5 is localized in a polar U-shape pattern facing the root tip of columella and root cap cells (Rigó et al., 2013). Studies show that *crk5* mutants treated by BFA had increased accumulation of PIN2 in BFA bodies. Hence, we can hypothesize that CRK5 influences PIN2 transport by either inhibiting endocytosis or activating recycling mechanisms.

MKK7 belongs to the MKKs family of kinases. It most likely acts as a positive transport regulator (Dai et al., 2006). Studies show that MKK3 and MKK6 probably act downstream from MKK7 (Jia et al., 2016). Interestingly, MKK7- MKK6 cascade shows to phosphorylate the S337 site of PIN1, thereby affecting PIN1 polarization and thus regulating the shoot branching (Jia et al., 2016). Further, it has been shown that the S337 site and its neighbour T340 site of PIN1 are essential for PIN polarity, for instance, in an embryo of *Arabidopsis thaliana* (J. Zhang et al., 2010). The phosphorylation of S337/T340 leads to depolarization of the PIN1 on the basal site of PM and redirection of the auxin flow, which is probably promoted by the MKK7- MKK6 cascade (Jia et al., 2016; J. Zhang et al., 2010).

2.5. Subcellular compartmentalisation

Subcellular compartmentalization of auxin metabolism and transport (Figure 5) is another level of intercellular auxin regulation. As mentioned in chapter 1.1.4. and 1.2.3., subcellular compartmentalization is essential for the regulation of plant developmental processes.

Auxin metabolism is primarily localized in the cytosol (Figure 2, 5). The first compartmentalization is the Trp synthesis, as it takes place in chloroplasts (Figure 2, 5). Moreover,

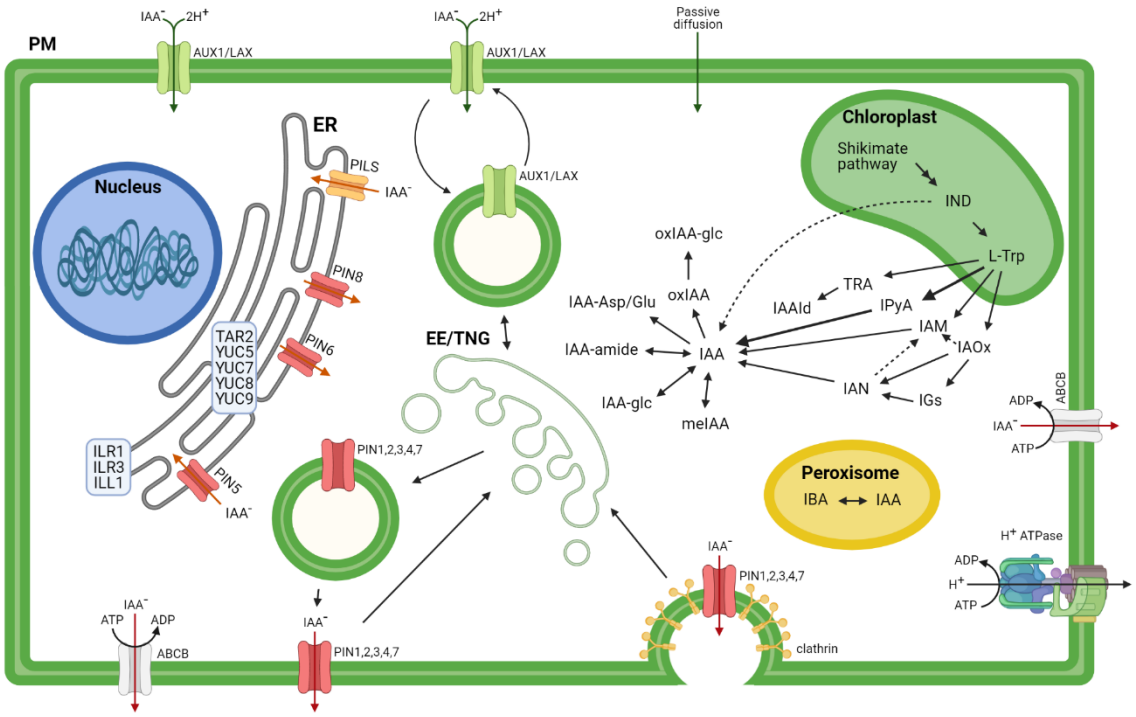

Figure 4. Subcelular compartmentation of Indole-3-acetic acid (IAA) transport and metabolism. Dashed arrows represent pathways, which are not well known or unknown. Solid arrows represent pathways, which genes, intermediates, or enzymes are studied. The shikimate pathway takes place in the chloroplast (green bean shape) (for review, see Maeda & Dudareva, 2012). IAA biosynthesis (Bak et al., 2001; Douglas Grubb et al., 2004; Gao et al., 2020; Ljung, 2013; Novák et al., 2012; Stepanova et al., 2008; Sugawara et al., 2009; Tao et al., 2008; Y. Zhao et al., 2001, 2002), IAA conversion to storage forms (Davies et al., 1999; Jackson et al., 2001; LeClere et al., 2002; Q. Liu et al., 2019; Ludwig-Müller et al., 2005; Ostrowski & Ciarkowska, 2021; Qin et al., 2005; Staswick et al., 2005; Y. Yang et al., 2008), and IAA catabolism (Novák et al., 2012; Östin et al., 1998; Porco et al., 2016; K. Tanaka et al., 2014; Z. Zhao et al., 2013) are shown. Bold arrows mark the main IAA biosynthesis pathway. The vice versa conversion of IBA to IAA takes place in the peroxisome (yellow oval) (Strader et al., 2010). Green are carriers from auxin-resistant/like aux family (AUX/LAX) (Carrier et al., 2008; Jonsson et al., 2017; Lomax et al., 1985). Red are carriers from pin-formed family (PIN) (Blilou et al., 2005; Dhonukshe et al., 2007; Geldner et al., 2003; Sauer et al., 2006). Orange are carriers from PIN-LIKES family (PILS) (Feraru et al., 2012). EE – early endosome; ER – endoplasmic reticulum; IAA-glc – IAA-glucose; IAAId – indole-3-acetaldehyde; IAM – indole-3-acetamide; IAN – indole-3-acetonitrile; IAOx – indole-3-acetaldoxime; IBA – indole-3-butyric acid; IGs – indole glucosinolates; ILLs – ILR1-like proteins; ILR1 – IAA-leucine resistant1; IPyA – indole-3-pyruvic acid; oxIAA – 2-oxindole-3-acetic acid; oxIAA-glc – 2-oxindole-3-acetic acid glucose; PM – plasma membrane; TNG- trans Golgi network; TRA – tryptamine; Trp – tryptophane; YUCs – YUCCAs.

the CYP79B2 and CYP79B3, which are components of the IAOx biosynthesis pathway, carry a chloroplast transit peptide(Hull et al., 2000). TAR2, YUC5, YUC7, YUC8, and YUC9 are localized on the ER (Figure 5) (Kriechbaumer et al., 2016). Also, ILR1, ILR3, and ILL1, responsible for the release of free IAA from IAA-amide, have been localized on the ER (Figure 5) (Sanchez Carranza et al., 2016). Interestingly, YUC4 has two different localizations. One is in the cytosol, and the

other on the cytosolic face of the ER membrane (Kriechbaumer et al., 2012). Hence, part of the IAA metabolism is probably dependent on the ER compartmentalization.

The ER localized PIN5, PIN6, PIN8, and PILs are essential for maintaining auxin homeostasis. They limit the amount of the cytosolic auxin flow to the nucleus, thereby regulate the auxin signalling responses. Suggesting that this is another example of the importance of subcellular compartmentalization in the auxin pathway.

Lastly, it has been shown that the distribution of the auxin afflux and influx carriers participate in the control of auxin organ-scale distribution and flux patterns, which, as mentioned above, is essential for plant development (Band et al., 2014; Blilou et al., 2005; Grieneisen et al., 2007).

3. Coordination of auxin transport and metabolism during plant development

Auxin, as the most studied phytohormone, is involved in various aspects of the plant development processes. For example, embryogenesis, root development, root meristem maintenance, organogenesis.

Plants respond to auxin minima and maxima. By low IAA levels, the topless (TPL) protein mediates an Aux/IAA repressor binding to the ARF localized on the auxin-regulated gene promoter sequences, resulting in suppression of the auxin-mediated response (Szemenyei et al., 2008). ARF can either act as an activator or a repressor of gene transcription, which is dependent on the type of ARF. Secondly, as a response to high IAA levels, IAA binds to auxin receptor TIR1, which is a part of the Skp, Cullin, F-box (SCF) complex. SCF complex is a ubiquitin ligase E3. Activated SCFTIR1 complex, by auxin, interacts with Aux/IAA, resulting in Aux/IAA ubiquitination, thereby activating the auxin-mediated response (Dharmasiri et al., 2005).

3.1. Embryogenesis

The embryogenesis starts after the fertilization of the ovule. After fertilization, in *Arabidopsis thaliana*, the zygote nucleus shifts to the apical pole. In this phase, the zygote begins to divide. The first division is asymmetrical. From the small apical cell will be almost the entire embryo generated. However, the suspensor, hypophysis, and root cap are formatted from the larger basal cell. The shoot apical meristem and root apical meristem are specified during the globular stage. The first lateral organs, which are cotyledons, are formatted during the heart stage. Later on, the embryo undergoes a pattern formation and morphogenesis. During the seed maturation of *Arabidopsis thaliana*, most of the endosperm is consumed, leaving the embryo surrounded with aleurone-like cell layer, which is next to the seed coat (Olsen, 2004).

It is well known that auxin regulates embryogenesis. Auxin builds up in immature seeds of *Arabidopsis thaliana* at the heart, torpedo, and cotyledon stages. Particularly at the ends of hypophysis and cotyledon primordia throughout the somatic embryo development (Ni et al., 2001). Also, auxin biosynthesis affects endosperm proliferation during seed growth and seed coat development (Figueiredo et al., 2015, 2016).

In *Arabidopsis thaliana*, early embryo development requires maternally produced auxin. Auxin biosynthesis is up-regulated in the integuments in the ovule after fertilization as the TAA1 levels gradually increase (Robert et al., 2018). Moreover, several transport carriers, like PINs, AUX1, LAX1, ABCB1, and ABCB19, were observed to express in the integuments (Robert et al., 2018). YUC1, YUC4, YUC10, and YUC11 are important in the globular stage of embryogenesis as they regulate auxin biosynthesis and thus modulate auxin levels (Cheng et al., 2007).

Further, auxin influx and efflux are required throughout the whole embryogenesis in the process of auxin-dependent cell specification (Robert et al., 2015) as polar auxin transport is responsible for the differentiation and definition of the embryonic tissues. In the embryo, *PIN1*, *PIN3*, *PIN4*, and *PIN7* were found to be expressed (Friml et al., 2003). It has been shown that PIN1 and PIN7 carriers form an apical-basal auxin gradient, and it specifies the apical and basal structures of the embryo (Friml et al., 2003). AUX1, LAX1, and LAX2 regulate the formation of the shoot and root poles (Robert et al., 2015), thus indicating that auxin efflux carriers also play an essential role in embryonic root formation, although the exact part of these influx carriers are still not completely comprehended.

3.2. Root development and root meristem maintenance

Auxin biosynthesis takes place primarily in the root apical meristem, where the genes for auxin biosynthesis are highly expressed (Brumos et al., 2018). Together with PAT, they establish the auxin levels in the root, which affects the root architecture. Local auxin biosynthesis probably depends on auxin transport as in lateral root formation with disruption of GNOM, the expression of YUC genes decreases (Guo et al., 2014).

YUC3, YUC5, YUC7, YUC8, and YUC9 are required for the high homeodomain-leucine zipper III (HD-ZIPIII) mediated xylem patterning (Ursache et al., 2014). Although PAT and auxin biosynthesis may have different roles in xylem patterning, they both are required for the proper patterning in *Arabidopsis thaliana* root during procambial development (Ursache et al., 2014). Also, YUCs regulate primary root and hypocotyl growth in response to a heat stress factor or aluminium (Franklin et al., 2011; G. Liu et al., 2016).

For the root growth, a small group of cells localized in the tip's centre are needed. These cells, called stem cell niche, coordinate the foundation of tissues in the root. Without maintaining the root meristem, the root loses its ability to grow, which is critical for the plant. Local auxin

maxima are required to maintain root meristem. As mentioned above, auxin is mainly produced in the root apical meristem, and the auxin made in the shoot is probably insufficient to meet the auxin levels required in the root (Qingguo Chen et al., 2014). Therefore, local auxin maxima in root stem cell niche are most likely established via the cooperation of local auxin biosynthesis and transport. This hypothesis supports the study of Brumos et al. (2018) as the study proposes that auxin transport from the shoot is indeed inefficient for maintaining root meristem, and thereby, the local auxin biosynthesis is indispensable. Altogether, this hypothesis suggests that under normal growth conditions, the cooperation of auxin biosynthesis and transport promotes root meristem maintenance. It also proposes the theory that local auxin biosynthesis most likely is able to maintain the auxin maxima by itself in unfavourable conditions (Brumos et al., 2018).

On the other hand, a study by Grieneisen et al. (2007) suggests that stem cell niche auxin maxima depend only on the polar-mediated PIN transport and the layout of the PIN carriers. The stable auxin maximum is accomplished by massive auxin flow through the tissue (Grieneisen et al., 2007). This flow consists of the central flow oriented downward to the root cap, where the flow is redistributed and continues in the epidermal cells upwards (Figure 4).

It is hard to determine which hypothesis is closer to the actual mechanism of the root meristem maintenance in plants. Further studies will be required to reveal the role of local auxin biosynthesis in this mechanism.

3.3. Leaf organogenesis

In the peripheral region of the shoot apical meristem, leaves development is initiated in three axes. These axes are adaxial-abaxial, proximal-distal, and medio-lateral. Three separate ways of leaves development have been proposed (Heisler & Byrne, 2020). One of them is by auxin flow establishment from primordium to the meristem. Low auxin levels promote the adaxial part of leaf formation (Qi et al., 2014). The low adaxial levels are established by auxin transport from leaves to the shoot apex (Qi et al., 2014). Further, wuschel related homeobox 1 (WOX1) and pressed flower (PRS) are probably expressed in the middle part of the leaf. Since they are not expressed in the adaxial part due to low auxin levels, and they are neither expressed in the abaxial part as they are silenced by repressor-type ARF (Guan et al., 2017).

In leaves formation, auxin biosynthesis also plays an important part. The suppression of several *YUC* genes results in narrow leaves, whereas overexpression results in curled leaves (Cheng et al., 2007; Kim et al., 2007). During leaf formation, adaxial-abaxial formation is not only established by PAT but also by auxin biosynthesis in leaf margin cells, suggesting that the leaf formation development is coordinated by auxin metabolism and transport (Wang et al., 2011). Moreover, local auxin biosynthesis is essential for vascular strand formation (Cheng et al., 2006).

Conclusion

This thesis began by noting that auxin as a phytohormone is an essential molecule for plant development and growth, and it is crucial to understand how auxin metabolism and transport are cooperating by these processes to comprehend the role of auxin in plants better. Indeed, the regulation mechanisms are complex and show that a single aberration can influence the development or growth process of the plant. However, in most cases, a plant reacts to the aberration by altering the developmental path and thus prevents itself from fatal consequences, making it harder to study each pathway separately and showing the plasticity of plants. Additionally, IAA is not the only molecule with auxin effects on plants, suggesting that auxin metabolism and transport mechanisms and their cooperation may be more complex and different in individual plant species.

The recent studies on this topic reinforce the importance of auxin metabolism and transport cooperation during plant development and growth, rather than acting separately. This idea is supported by the fact that auxin metabolism and transport are frequently regulated together and can regulate each other as well. Moreover, each individual cell precisely regulates auxin homeostasis by modulating both auxin metabolism and transport. Hence, cells react to both low and high auxin levels with different responses by trying to maintain the auxin homeostasis. However, most of the studies about auxin are aimed only at one of auxin metabolism or transport in o role of plant development. This may lead to incorrect interpretations of the plant's developmental pathways, which are complex methods requiring the cooperation of not only auxin metabolism and transport but also other phytohormones, molecules, and many additional factors.

Even though to this day there are just a few studies that address the cooperation of auxin metabolism and transport, and it is a relatively new conception, the development of new and more precise mechanisms to follow up auxin transport and metabolism *in vivo* will help further studies to observe and understand these phenomena and its importance in the context of plant growth and development.

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