Auxin biosynthesis: spatial regulation and adaptation to stress

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Running title: TAA/YUC-dependent auxin biosynthesis

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Highlight section

This review highlights recent advances in TAA/YUC-dependent auxin biosynthesis focussing on subcellular localisation of auxin biosynthetic enzymes, differential regulation in root and shoot, and the influence of abiotic stress.

Abstract

The plant hormone auxin is essential for plant growth and development, controlling both organ development and overall plant architecture. Auxin homeostasis is regulated by coordination of biosynthesis, transport, conjugation, sequestration/storage, and catabolism to optimize concentration-dependent growth responses and adaptive responses to temperature, water stress, herbivory and pathogens. At present, the best defined pathway of auxin biosynthesis is the TAA/YUC route, in which the tryptophan aminotransferases TAA and TAR and YUCCA flavin-dependent monooxygenases produce the auxin indole-3-acetic acid from tryptophan. This review highlights recent advances in our knowledge of TAA/YUC-dependent auxin biosynthesis focussing on membrane localisation of auxin biosynthetic enzymes, differential regulation in root and shoot tissue, and auxin biosynthesis during abiotic stress.

Keywords: auxin biosynthesis, membrane, endoplasmic reticulum, YUC, TAA, TAR, halotropism, metabolon, heat stress, drought.

Introduction

Auxin is a central plant growth regulator involved in almost all aspects of plant growth and development, as well as responses to external stimuli including both biotic and abiotic

stresses. The critical role of auxins in regulating plant form and function has resulted in the use of synthetic auxins and auxinic compounds to achieve various processes in agriculture and horticulture, including the stimulation of root growth in cuttings, the promotion of fruit production, and the killing of broadleaf weeds by auxinic herbicides such as 2,4-dichlorophenoxyacetic acid [2,4-D] or dicamba. The most abundant auxin, indole-3-acetic acid (IAA), affects almost all aspects of plant development including apical-basal polarity (Friml *et al*., 2003), root formation (Benková *et al*., 2003), stem elongation, and tropic growth responses. Auxin homeostasis is regulated by coordinating the biosynthesis, transport, conjugation, storage, and catabolism of IAA to optimize concentration-dependent growth responses and adaptive responses to temperature, light conditions, gravity, water stress, oxidation, herbivory, and pathogens. For example, high temperatures alter local auxin levels in the shoot apex and negatively impact yield in maize, wheat, barley and tomato (Higashitani, 2013). The spatial patterning of auxin accumulation at the tissue level is generated by a combination of localised auxin biosynthesis and both non-polar (in areas of high auxin concentration) and polar transport across cells. This results in the formation of auxin gradients throughout the plant. Both roots and shoots exhibit linear gradients of auxin across the longitudinal axes of these organs, in which auxin levels are generally most concentrated in the organ meristems and rapidly dividing tissues (such as young leaves or root primordia) and less concentrated in more mature, expanded tissues (Kramer and Bennett, 2006). However, external stimuli, such as light, gravity, or directional salt stress, can also result in the formation of lateral auxin gradients across the transverse axes of root and shoot tissues, resulting in directional (tropic) growth of these organs. Finally, external stress stimuli, such as heat, drought, UV, insect feeding/tissue damage, and pathogen attack, can also result in the formation of auxin gradients centred on the primary site at which the stress occurs. These IAA gradients result in dose-dependent adaptive growth responses (Bhalerao and Bennett, 2003), which occur as increasing amounts of auxin are perceived by the TIR/AUX family of auxin receptors (Dharmasiri *et al*., 2005).

Given the essential role of auxin gradients in regulating plant growth and responses to the environment, it is not surprising that, to date, much research in the area of auxin biology has focused on the mechanisms responsible for creating these gradients. These efforts have resulted in the elucidation of the chemiosmotic model of auxin transport (reviewed in Blakeslee *et al*., 2005; Peer *et al*., 2011; Han *et al*., 2017), as well as many of the proteins responsible for facilitating the transport of auxin into and out of cells. The chemiosmotic model of auxin transport is dependent upon the generation of a proton (and pH) gradient by plasma membrane ATPase proteins. Accumulations of protons in the apoplast decrease the pH of this compartment relative to the cytosol, resulting in the protonation of extracellularly-localized IAA (a weak acid; pKa 4.75) molecules. Protonated auxin molecules enter the cell

via diffusion and the action of AUX/LAX IAA-proton symport transporters. Inside the cell, exposure to the more neutral pH conditions de-protonates IAA molecules. Deprotonated IAA- ions can no longer diffuse through membrane, and can only exit the cell via the activity of transport proteins. Previous work has demonstrated that both PIN-formed (PIN) major efflux facilitator and ATP-Binding Cassette type B (ABCB)/p-glycoprotein (PGP) proteins are the primary transporters responsible for auxin efflux from cells (Han *et al*., 2017). A large body of research has supported a role for asymmetrically-localized PIN transporters in the directional transport of auxin from cells; while ABCB proteins generally exhibit a more apolar localization and use energy (ATP) to transport IAA against a localized gradient in areas of high auxin concentration (Han *et al*., 2017; Peer *et al*., 2011).

While much is known about auxin transport by influx and efflux carriers, important questions regarding the mechanisms controlling IAA homeostasis (e.g. conjugation and transport) and the nature of auxin biosynthetic pathways remain unanswered (Chandler, 2009). This is partly due to the complexity of the multiple pathways (on both the synthesis and catabolism sides of the homeostasis "equation") forming the IAA metabolic network that changes dynamically to maintain homeostasis or to supply auxin for local demands. Depending on the organ, developmental stage or environment (Normanly *et al*., 1999; Östin *et al*., 1999), parallel tryptophan-dependent and -independent pathways (Kriechbaumer *et al*., 2006; Woodward and Bartel, 2005) may be differentially regulated and form a metabolic network that changes dynamically to maintain homeostasis or to supply IAA for local demands. Many previously published studies also presumed that auxin is ubiquitously and continuously synthesized in all plant organs, and that gradient formation via transport alone is sufficient to initiate and maintain auxin action in developmental processes and responses to external stimuli. However, recent work has shown though that there is definitely more to auxin biosynthesis; and that auxin metabolism is both spatially and temporally regulated (Zhao, 2010; Robert *et al*., 2018; Yao *et al*., 2018; Brumos *et al*., 2018). To understand the mechanism of local auxin action and gradient formation it is essential to determine how and where auxin biosynthesis is regulated.

Currently, the most well-defined pathway of auxin biosynthesis is the highly-conserved TAA/YUC route, in which tryptophan aminotransferases and YUCCA (YUC) flavin-dependent monooxygenases produce the auxin IAA from tryptophan. The two-step pathway converting tryptophan (Trp) to IAA catalysed by the Tryptophan Aminotransferase of Arabidopsis (TAA) and the YUC flavin-containing monooxygenases is the first identified complete auxin biosynthetic pathway, and to date has been found to be essential for almost all of the major developmental events in plants (Zhao, 2014). The essential nature of IAA synthesis is emphasized by the fact that each of the genes of the TAA/YUC pathway are members of multi-gene families, with 11 *YUC* genes and 3 *TAA/TAR* genes in present in Arabidopsis,

giving this important pathway a high level of functional redundancy. As the TAA/YUC pathway is currently the best-defined route of auxin synthesis in plants, it is worth detailing the chemical reactions of this pathway in more detail. Following the synthesis of the amino acid tryptophan via the shikimic acid pathway, the TAA/TAR enzymes convert Trp to indole-3-pyruvic acid (IPyA) (Mashiguchi *et al*., 2011; Philipps *et al*., 2011; Kriechbaumer *et al*., 2012). Inactivation of the first step in TAA/YUC-dependent auxin biosynthesis (via knockout of *TAA1* or its close homologs *TAR1* and *TAR2*) results in global reductions in levels of endogenous auxin, leading, ultimately, to defects in several developmental processes (Stepanova *et al*., 2008). The IPyA generated by the TAA enzymes is then converted to IAA by YUC monooxygenase genes. YUC enzymes were first identified as key auxin biosynthesis enzymes in Arabidopsis thaliana, where overexpression of members of the *YUCCA* gene family leads to auxin overproduction (Zhao *et al*., 2001). YUC enzymes were identified via the first ever auxin biosynthetic mutants (Cheng *et al*., 2006; Kim *et al*. 2007).

While research over the past decade has provided an increasingly clear picture of the genes and enzymes involved in auxin synthesis, several questions regarding the biochemical mechanisms and subcellular localization of this process remain unanswered. In this summary we review some of the recent findings and novel avenues taken in researching auxin biosynthesis via the TAA/YUC pathway (Box 1). Specifically, we focus on recent advances in the areas of cellular imaging, protein tagging, and metabolite quantification which have provided a better understanding of where IAA synthesis is localized in cells, how this synthesis is regulated, and the rate of auxin precursors and degradation products.

Location, location, location

Membrane localisation of YUC-dependent auxin biosynthesis

Most previous research on auxin synthesis has been focused on the tissue and cell level. In many of these studies, indirect visualization tools, primarily auxin-sensitive gene constructs such as DR5:GUS or D2:VENUS, have been used to highlight cells or tissues accumulating auxin (reviewed in Blakeslee and Murphy 2016). As these studies use tools that have a multi- or single-cell limit of resolution, they have not been able to provide information regarding the sub-cellular sites of auxin synthesis. More recent work, however, has uncovered novel control mechanisms in the TAA and YUC gene families that involve location of auxin biosynthetic enzymes to the endoplasmic reticulum (ER) and tissue-specific gene splicing (Kriechbaumer *et al*., 2012), which have major consequences for functional compartmentation. Membrane association raises the intriguing possibility that auxin synthesis is compartmentalised in specific sub-cellular organelles and/or membranes. Membrane localisation of auxin biosynthetic enzymes was first described for the Arabidopsis YUC4 enzyme. YUC4 exists in two tissue-specific alternatively spliced isoforms capable of

converting IPyA to IAA (Kriechbaumer *et al*., 2012). Due to isoform-specific splicing and alternative *3*' end processing of mRNAs, the flower-specific YUC4.2 isoform features a strong C-terminal hydrophobic transmembrane domain (TMD) and is located on the ER membrane, with its N-terminal enzymatic domain facing the cytosol (Kriechbaumer *et al*., 2012). In contrast, the *YUC4.1* isoform is transcribed ubiquitously, and the YUC4.1 protein exhibits a cytosolic localization. In both maize and Arabidopsis, about half the enzymes in both the TAA/TAR and YUC enzyme families are localised to the ER membrane (Kriechbaumer *et al*., 2015, 2016; Figure 1). In maize *Zm*TAR1 and the YUC orthologue *Zm*SPI1 are localised to the ER membrane; while in Arabidopsis YUC3, 5, 7, 8, 9 and TAR2 are ER-localised. The hypothesis that these enzymes are actively involved in auxin synthesis is supported by the fact that in maize primary root and coleoptile (Kriechbaumer *et al*., 2015), as well as in Arabidopsis seedlings (Kriechbaumer *et al*., 2016), about 20% of the total auxin biosynthetic activity was detected in a purified microsomal membrane fraction containing ER membranes but not plasma membrane or mitochondrial membranes (Kriechbaumer *et al*., 2015, 2016).

Interestingly, there is increasing evidence that the presence of IAA in the ER can alter the structure of this organelle, particularly in leaves, and potentially influence ER-localized auxin synthesis. For example, when IAA is infiltrated into tobacco leaves together with the ER marker GFP-HDEL, the network structure of the ER in epidermal cells is quite drastically perturbed, resulting in general misalignment of tubules as well as induction of ER cisternae (Figure 2). This ER-disruption phenotype is also observed when IAA biosynthetic enzymes carrying out the first (TAA/TAR) and second step (YUC) of the biosynthesis are coexpressed in tobacco leaf mesophyll cells (Figure 1C), potentially resulting in auxin hyperaccumulation (Kriechbaumer *et al*., 2016). This phenomenon could indicate that auxin regulation is also of great importance for ER functionality. Further, these data provide further insights into additional mechanisms by which auxin may regulate its own biosynthesis.

The ER serves not only as a platform for auxin biosynthesis, but also as auxin recycling station (Friml *et al*., 2003). Both PIN and PIN-Like (PILS) auxin transporter proteins are present on the ER membrane (Mravec *et al.*, 2009; Barbez *et al.*, 2012; Dal Bosco *et al.*, 2012; Ding *et al.*, 2012; Bender *et al.*, 2013; Sawchuk *et al.*, 2013), indicating that cytosolic and/or endosomally localized auxin can be transported into the ER. Additionally, the auxin-deconjugating enzymes ILL2, IAR3 and ILR1 have been shown to reside in the ER, where they are likely to increase the content of active IAA by amido-hydrolysis of IAA-amino acid conjugates (Sanchez *et al*., 2016). To date, however, the technical difficulties involved in reliably extracting intact sub-cellular organelles has made it difficult to reliably quantify

localized auxin concentrations at the sub-cellular level (*i.e.,* in or around the ER) using biochemical or mass spectrometric techniques (Blakeslee and Murphy, 2016; Blakeslee, unpublished).

Up or down - root or shoot makes a difference

It has long been suggested that the initial pulse of auxin required for embryonic/early root development is produced in shoots and transported to the root via polar auxin transport (Benkova *et al*., 2003; Grieneisen *et al*., 2007). However, as the root develops, a burst of shoot-derived auxin triggers auxin synthesis in the root apical meristem, and this phenomenon (a localized accumulation of shoot-derived auxin leading to initiation of auxin synthesis) is recapitulated in lateral root meristems (Casimiro *et al*., 2001). These data highlight the importance of local auxin biosynthesis; and more recent studies have provided evidence that specific combinations of auxin synthesis enzymes are responsible for tissue-specific IAA synthesis. Different combinations of knockouts of individual *YUC* genes affect different developmental processes and tissues; and these mutant phenotypes cannot be complemented by expression of the YUC proteins found in other tissues (Zhao, 2018). Correct organ development requires specific YUC-TAA/TAR protein combinations that have spatially and temporally similar expression and expression patterns of different TAA and YUC proteins (reviewed in detail in Zhao, 2018). In general, Arabidopsis appears to use two separate sets of YUC genes for auxin biosynthesis in roots and shoots. Gene and protein expression data indicate that YUC1, 2, 4 and 6 are the main YUCs functioning in shoots, while YUC3, 5, 7, 8 and 9 are responsible for producing auxin in roots (Chen *et al*., 2014). This hypothesis is supported by mutant studies: *yuc1/2/4/6* quadruple mutants have severe defects in vascular patterning and flower development, but have no root defects, consistent with their shoot-localized expression patterns (Chen *et al*., 2014). YUC3, 5, 7, 8 and 9 are expressed during root development and quintuple mutants of these five YUC genes (*yucQ* mutants) develop short, agravitropic roots (Chen *et al*., 2014). These *yucQ* root phenotypes are rescued by the addition or exogenous IAA or the expression a *YUC* gene in *yucQ* roots; but not by overexpression of a *YUC* gene in shoots (Chen *et al*., 2014). These data provide further support for the essential role of root-derived auxin in normal root growth and development.

Interestingly, shoot- and root-localized YUC proteins appear to exhibit differential, organ-specific sub-cellular localization patterns. The shoot YUCs 1, 2, 4.1 and 6 are cytosolically localized, whereas the YUCs expressed in roots (YUC3, 5, 7, 8 and 9) are ER membrane-bound (Kriechbaumer *et al*., 2016; Poulet and Kriechbaumer, 2017; Figure 3). In contrast to YUC proteins, Arabidopsis seedlings appear to use the same set of *TAA/TAR* genes in both roots and shoots (Stepanova *et al*., 2008; Tao *et al*., 2008). Based on these data, it can be

hypothesized that ER-bound auxin biosynthesis is likely to be predominant in roots (based on the ER localization of the YUC proteins), while cytosolic IAA synthesis may be the dominant mechanisms in shoots. It is possible that specific YUC isoforms, with their individual sub-cellular localizations in the root (membrane) vs. the shoot (soluble) are needed due to the distinct impact of auxin on the actin cytoskeleton and ER structure of cells in root vs. shoot tissues. For example, while in shoot tissues auxin treatments result in increased actin-dependent trafficking and streaming, roots exhibit decreased actin streaming and polymerization (Rahman *et al*., 2007; Sparkes *et al*., 2009). However, as noted above, difficulties in quantifying auxin levels with sub-cellular level resolution have to date made it difficult to determine both localized internal auxin concentrations and the impact of sub-cellular auxin gradients on cellular structure and membrane trafficking.

Auxin biosynthesis during abiotic stress

Environmental conditions such as high salinity, drought, chilling, and metal toxicity cause severe stress to plants, negatively impacting their growth, development, and production yields (in the case of crop species). The increasing effects of climate change on weather patterns have resulted in plants being increasingly exposed to both extremes of temperature (heat/cold) and water stress (drought/salt) (reviewed in Han *et al*., 2017). To date, the majority of the work done to investigate the role of auxin in abiotic stress responses has focused on water-related stresses, particularly salt, drought, and metal ion stresses. It is estimated that ~20% of the world's arable soil is currently experiencing salt stress, while 28% of global farmland is too dry for crop production (Blumwald, 2000).

Auxin responses to salt stress

Saline contamination of soils is usually the result of the use of contaminated water, fertilizer aggregation in soils following repeated rounds of irrigation and drying, or the intrusion of seawater into agricultural systems (in coastal areas) (Blumwald, 2000). Sodium chloride, the salt most commonly responsible for saline stress in soils, exerts both osmotic and ionic stress on plant tissues (Tester and Davenport, 2003; Munns and Tester, 2008). Sodium chloride, however, is not the only salt inducing plant stress responses. Increased use of contaminated, marginal, or reclaimed water supplies has also resulted in increased exposure of plant roots to metal salts ions (aluminium, copper, etc.). Further, while most studies have focused on root tissues, salt stress can also occur on leaves, for example during herbicide drift. Plant adaptive responses to saline stress fall into three broad categories: transport of NaCl out of the cytosol, exclusion of salt from aerial tissues and extrusion from root tissues, and adaptive/tropic growth of root systems (Shi and Zhu, 2002; Zhu 2002, 2003; Julkowska and Testerink, 2015; reviewed in Han *et al*., 2017). Recent work has demonstrated that plant

adaptive growth responses following salt stress are dependent upon the tightly regulated synthesis and transport of the phytohormone auxin (reviewed in Han *et al*., 2017). During salt stress, auxin functions to regulate key aspects of root architecture, including the regulation of root elongation (in coordination with the abscisic acid), the initiation of lateral root growth, and tropic growth (Julkowska and Testerink, 2015). While auxin has a well-defined role in regulating gravitropic responses (growth of the root in the direction of the gravity vector), only more recently has auxin been implicated in regulating halotropic responses, or the directional growth of roots away from areas of high salinity (Galvan-Ampudia, 2013). In gravitropism lateral accumulations of auxin occur on the side of the root closest to the gravitational stimulus, resulting in directional growth towards the gravity vector; in contrast halotropism results in lateral accumulations of auxin on the side of the root furthest from the salt stimulus, resulting in directional growth away from the area of increased salinity (Galvan-Ampudia, 2013; Rosquete and Kleine-Vehn, 2013; van den Berg *et al*., 2016). Halotropism has been demonstrated to be dependent upon both the tightly regulated activity of phosphatidic acid produced primarily by phospholipase D (PLD) enzymes; and auxin transport mediated by AUX/IAA influx symporters and PIN2 efflux transporters (Sun *et al*., 2008; Galvan-Ampudia, 2013; Rosquete and Kleine-Vehn, 2013; van den Berg *et al*., 2016; reviewed in Han *et al*., 2017). To date the role of IAA metabolism in this process has not been well determined. However, several lines of evidence support a role for modified auxin metabolism in salt stress responses. For example, in several species flavonoids, which prevent auxin catabolism (likely as a result of their anti-oxidant activity), have been demonstrated to increase salt tolerance (van Oosten *et al*., 2013; Yan *et al*., 2014).

TAA/YUC responses to aluminium contamination

While previous work has indicated that decreasing IAA catabolism increases salt stress tolerance, other studies have implicated the upregulation of the auxin biosynthetic pathway in several abiotic water-related stress responses. For example, the root specific *YUCs 3*,*5*,*7*,*8* and *9* have been reported to mediate root growth inhibition induced by aluminium stress in Arabidopsis plants (Liu *et al*., 2016). These results were supported by further work in which inhibition of root growth in response to aluminium toxicity was found to be dependent upon activation of the auxin biosynthetic pathway, specifically by the upregulation of *TAA1* (Yang *et al*., 2014).

Heat and cold stress can induce specific *YUC* genes

Heat shock treatments in plants have been shown to induce ER sheet formation (Pain *et al*., 2019). In cucumber CsYUC8 and 9 are upregulated under high temperatures (38°C),

resulting in elevated IAA levels (Yan *et al*., 2016). Interestingly, heat stress (a common cause of salt/drought/water stress) induced similar responses in Arabidopsis, where exposure to high temperatures resulted in increased expression of YUC8 activated by the transcriptional regulator phytochrome-interacting factor 4 (Sun *et al*., 2012). Cold stress also resulted in altered auxin synthesis in cucumber, with temperatures of 4°C upregulating CsYUC10b while downregulating the other YUC proteins (Yan *et al*., 2016).

Analysis of Arabidopsis RNAseq data collected during heat and drought stress responses (https://www.ncbi.nlm.nih.gov/geo/) shows tissue-specific differences in up- or downregulation of *TAA/YUC* auxin biosynthetic genes (Figure 4A). The most prominent responses observed in these data sets were the upregulation of *YUC9* expression in leaf tissue after heat stress, and the strong down-regulation of both *TAA1* and *TAR2* transcription levels in leaves following drought stress (Figure 4B).

Do YUC genes improve water stress tolerance?

Several studies have provided evidence that highly regulated auxin synthesis is essential for adaptive growth during longer-term drought stresses. For example, increased IAA levels have been linked to enhanced tolerance to drought conditions in alfalfa (Defez *et al.*, 2017). Overexpression of *YUC7* in Arabidopsis (Lee *et al*., 2012), and *YUC6* in both potato and Arabidopsis also resulted in increased drought tolerance (Kim *et al.*, 2013, Cha *et al.*, 2015). Interestingly, the increased drought tolerance following *YUC6* overexpression was determined not to be a result of increased auxin synthesis, but instead due to an additional thiol-reductase activity found in YUC6, which would diminish ROS activity and IAA catabolism (Cha *et al.*, 2015). In other words, in this case overexpression of YUC6 was thought not to increase IAA synthesis, but instead decrease IAA catabolism; this is similar to the mechanism proposed for flavonoids above. In Arabidopsis, however, increased levels in IAA have been thought to directly increase drought tolerance through a variety of mechanisms including: modification of root architecture, regulation of the expression of ABA synthesis genes, and alteration of metabolic pathways (production of ROS etc; Shi *et al.*, 2014). An increase in IAA levels in roots in response to drought stress has also been observed in two commercial tomato species, which has been proposed to increase tolerance via increasing root biomass/surface area—most likely through increasing the number of lateral roots present (Moles *et al.*, 2018). In contrast to shoots/aerial tissues, which generally contribute to water loss, roots contribute to drought adaptation by producing specialized molecules and modulating growth to simultaneously maximize surface area and extract water out from ever-drier soils (Davies *et al*., 2002).In contrast to most results in Arabidopsis and other species, some studies in rice have associated increased drought tolerance with decreased levels of IAA in roots, and found that various *YUC* genes were down-regulated

under these conditions (Zhang *et al.*, 2009; Du *et al.*, 2013; reviewed in Nasser and Shani, 2016).

While previous work has focused on the involvement of auxin in water/heat/ion abiotic stress responses in roots and shoots, little is known about the mechanisms by which plants perceive these stresses, and the role of auxin in this process. In addition to serving as water uptake interfaces, roots also act as primary sensors to detect water shortage (Davies *et al*., 2002). Interestingly, the root-shoot transition zone is emerging to be a potential focal point for *YUC*-dependent regulation of salt/drought/ionic stress responses. Positioned at the intersection of aerial and root tissues, the TZ has been increasingly implicated in the perception of environmental stresses and hormonal cross-talk following stimuli (Kong *et al*., 2018). Finally, analysis of RNAseq data (Genevestigator) indicates that transcription levels of the ER-localised root *YUC* genes are increased nearly 2fold in roots (data not shown) but not in leaves (Figure 5B) during drought stress which could make them interesting candidates for increasing drought tolerance.

Outlook: To boldly go…

The past decade has seen radical advances in the area of auxin biochemistry, transport, and physiology. The mechanisms of auxin signalling, transport/gradient formation and metabolism (synthesis, catabolism, and conjugation) are being increasingly well-defined. Despite this progress, however, several questions remain regarding the means by which a single simple molecule such as auxin is critical for nearly all aspects of plant life. In this review we have focused on recent developments in the field of YUC-dependent auxin synthesis. The tissue and cellular specificity of YUC proteins allows for cell- and organ-specific auxin biosynthesis throughout development or in response to external stimuli.

The growing list of auxin-related proteins located at the ER membrane in multiple plant species indicates that compartmentation of auxin synthesis may be a general occurrence in the evolution of higher plants. Thus, a comprehensive analysis and mapping of the subcellular location of auxin enzymes including enzymes involved in auxin transport, conjugation and deconjugation would be of great value. ER membrane-anchoring indicates a compartmentation and level of organization which has not previously been suspected or demonstrated for auxin biosynthetic enzymes. Since many of the enzymes potentially involved in auxin biosynthesis have low substrate specificities and turnover rates, metabolic channelling in an IAA synthase complex has been postulated (Müller and Weiler, 2000). Multi-enzyme metabolic pathways, particularly those consisting of membrane-localized proteins, can be organized into metabolons, which enable the highly coordinated and regulated processes involved in synthesis or catabolism to be carried in an efficient manner, increasing throughput and yield (Møller, 2010). Metabolons will typically be comprised of (i)

sequential enzymes in the pathway (e.g. TAA/TAR, YUC) together with (ii) scaffolding proteins allowing for efficient channelling of metabolic intermediates from one active site to the next (Jørgensen *et al*., 2005). Such a mechanism increases local substrate concentration and turnover rates, prevents unwanted diffusion and metabolic interference, and is beneficial for containment of labile or toxic intermediates; for example the auxin precursor IPyA is highly unstable in water (Kriechbaumer *et al*., 2015). Candidate proteins for such scaffolding are chaperones and/or membrane-anchored cytochrome P450 enzymes that can serve as nucleation points and platforms for metabolon formation (Jørgensen *et al*., 2005). Hence metabolons also can comprise cytosolic proteins that can bind or temporally interact with membrane-bound proteins or platform and scaffolding proteins such as the P450 enzymes. One final set of components suggested to play a role in metabolon formation (iii) are membrane-structural proteins and highly structured membrane sub-domains (i.e. detergent resistant membrane microdomains); for example, P450 enzymes involved in a lignin biosynthetic metabolon were co-purified with reticulon proteins (Bassard *et al*., 2012) and specific membrane sub-domains have been shown to be important in maintaining multi-protein complex integrity in plant, mammalian, and yeast cells. In plants, metabolons have been shown to play a role e.g. for the production of flavonoids (Hrazdina and Wagner, 1985) and sporopollenin (Lallemand *et al*., 2013) in Arabidopsis, as well as the glucoside dhurrin (Nielsen *et al*., 2008; Laursen *et al*., 2016) in sorghum. More recently a soybean isoflavonoid metabolon tethered to the ER has been reported (Dastmalchi *et al*., 2016). The area of metabolon research is therefore of great interest for the regulation and manipulation of the synthesis of not only auxin but also for a range of metabolites and secondary compounds.

All in all, there is no end to the baffling complexity and the plethora of effects mediated by auxin. Even as our knowledge in the areas of auxin regulation/signalling, biosynthesis, transport, conjugation, sequestration/storage, and catabolism has increased, one question still remains: how a simple single molecule like auxin can be crucial for nearly all aspects of plant life?

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Box 1. Key developments in understanding TAA/YUC-dependent auxin biosynthesis

- **Local auxin biosynthesis is critical in the formation of localised auxin gradients, as well as early embryo patterning and flower fertility**

  Recent research, including that presented in Brumos *et al*. (2018) and Robert *et al*. (2016), suggests that local auxin biosynthesis plays an important role in the formation of localised auxin gradients and is crucial in early embryo patterning (Robert *et al*., 2016) and flower fertility (Brumos *et al*., 2018). In these processes, it is likely that auxin synthesis and transport act synergistically, as has been demonstrated for root meristem maintenance (Brumos *et al*., 2018).

- **Plant metabolons allow for efficient synthesis and complex regulation of crucial plant products and can take advantage of cellular compartmentalisation.**

  Laursen *et al*. (2016) and Dastmalchi *et al*. (2016) are key recent papers describing plant metabolon complexes. Laursen *et al*. (2016) presents the composition of a dynamics metabolon complex catalyzing the formation of a sorghum defense compound, the cyanogenic glucoside dhurrin, as well as the importance of the lipid composition for metabolon formation. Data presented in the Dastmalchi *et al*. (2016) indicate that the soybean isoflavonoid metabolon is also tethered to the endoplasmic reticulum.

- **Auxin and abiotic stress are closely linked**

  There is increasing evidence of the link between abiotic stresses and auxin (as well as other plant growth regulators). The role of auxin in regulating halotropic growth is well-summarized and modelled in van den Berg *et al*. (2016); and a prospective mechanism of how auxin metabolism may impact this process is provided in Han *et al*. (2017).

Figure legends

Figure 1: Localisation of TAA/YUC proteins. Tobacco leaf epidermal cells are expressing different combinations of TAA/YUC proteins tagged to fluorescent proteins. Cells are imaged using confocal microscopy (Zeiss LSM880 with Airyscan detector). YUC5-mCherry, YUC9-GFP, TAR2-mCherry are ER-localised. GFP-TAA1 is cytosolic. Expression of the whole IAA synthetic pathway (C) often results in an ER network with enhanced cisternal structures. Scale bar = 5µm

Figure 2: ER network with increasing auxin concentrations. Tobacco leaf cells are infiltrated with the ER-marker GFP-HDEL and varying amounts of IAA. Cells are imaged using confocal microscopy (LSM880 with Airyscan detector) with increasing amounts of IAA the ER network structure gets more perturbed. Two representative images (upper and lower panel) are shown per IAA concentration. Scale bar = 5µm.

Figure 3: Graphic representation of arabidopsis YUC proteins for root-shoot transcription as well as subcellular localisation (cytosol and endoplasmic reticulum – ER).

Figure 4: Up-or downregulation of TAA/TAR and YUC transcripts after abiotic stresses. RNA sequencing transcript analysis for TAA/TAR and YUC proteins after (A) heat stress in seedlings 21 days after germination (grey bars) and adult leaves (blue bars) or (B) drought stress in leaves, respectively. Sequence Read Archives (SRA) files were used for RNA sequencing analysis. All SRA files were downloaded from http://www.ncbi.nlm.nih.gov/geo/browse/. (Control drought: SRR921311, SRR921312; Drought stress: SRR921315, SRR921316; Control heat seedlings: SRR2033948, SRR2033949; Heat stress seedlings: SRR2033950, SRR2033951; Control heat stress leaves: SRR1020621; Heat stress leaves: SRR1020622). Control and stress data were calculated as RPKM (reads per kilobase of transcript per million mapped reads), was normalised to the reference gene *AtSAND* (At2g28390) and finally values for control conditions were deducted from the values for the stress condition to indicate up- or downregulation of transcripts.

Figure 1

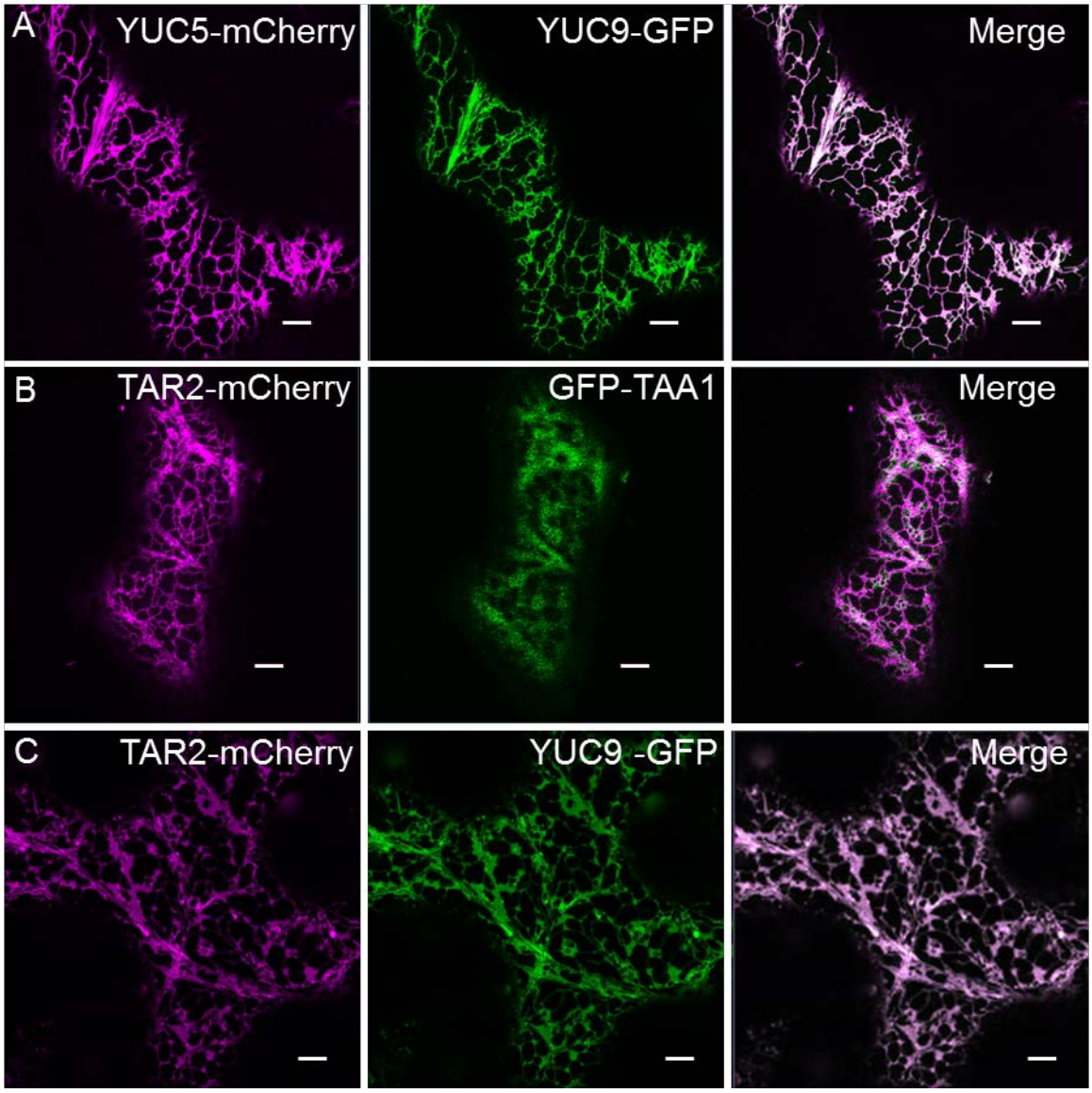

A
YUC5-mCherry
YUC9-GFP
Merge
B
TAR2-mCherry
GFP-TAA1
Merge
C
TAR2-mCherry
YUC9 -GFP
Merge

Figure 2

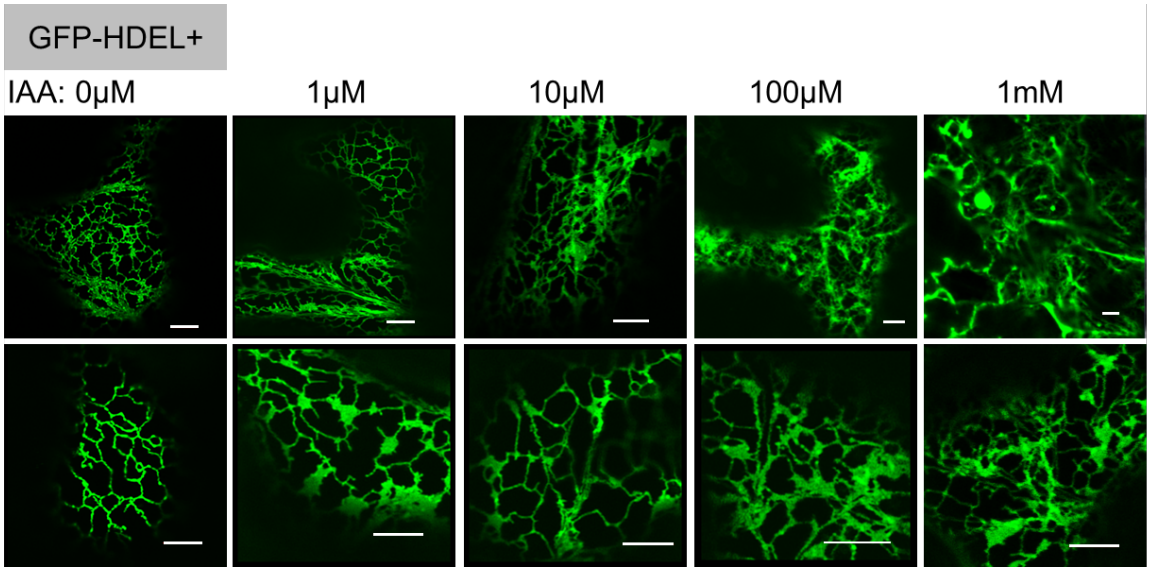


Figure 3

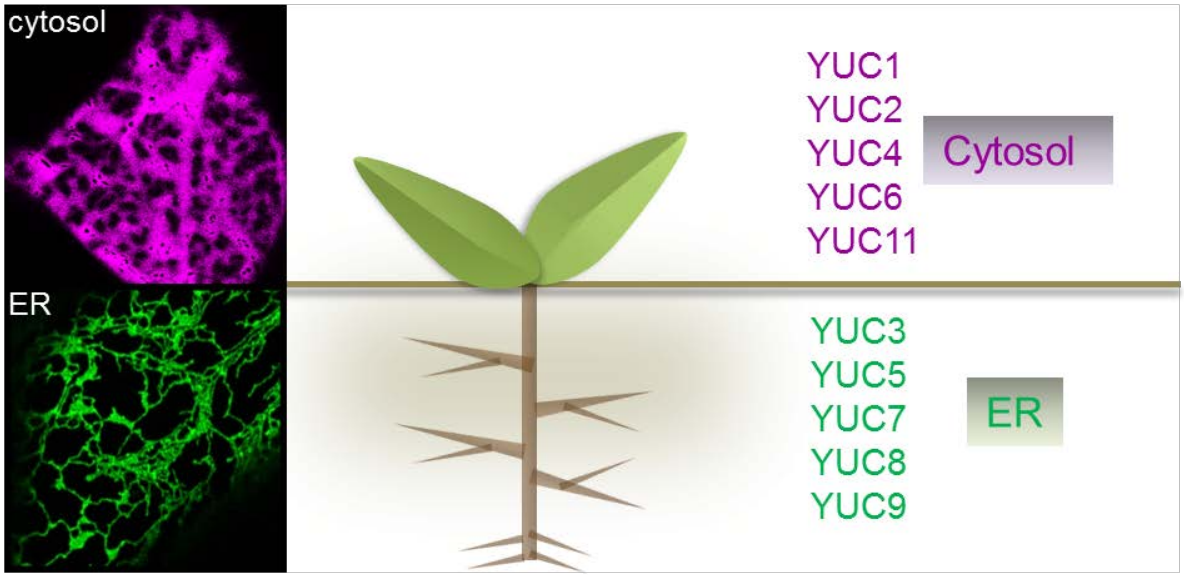
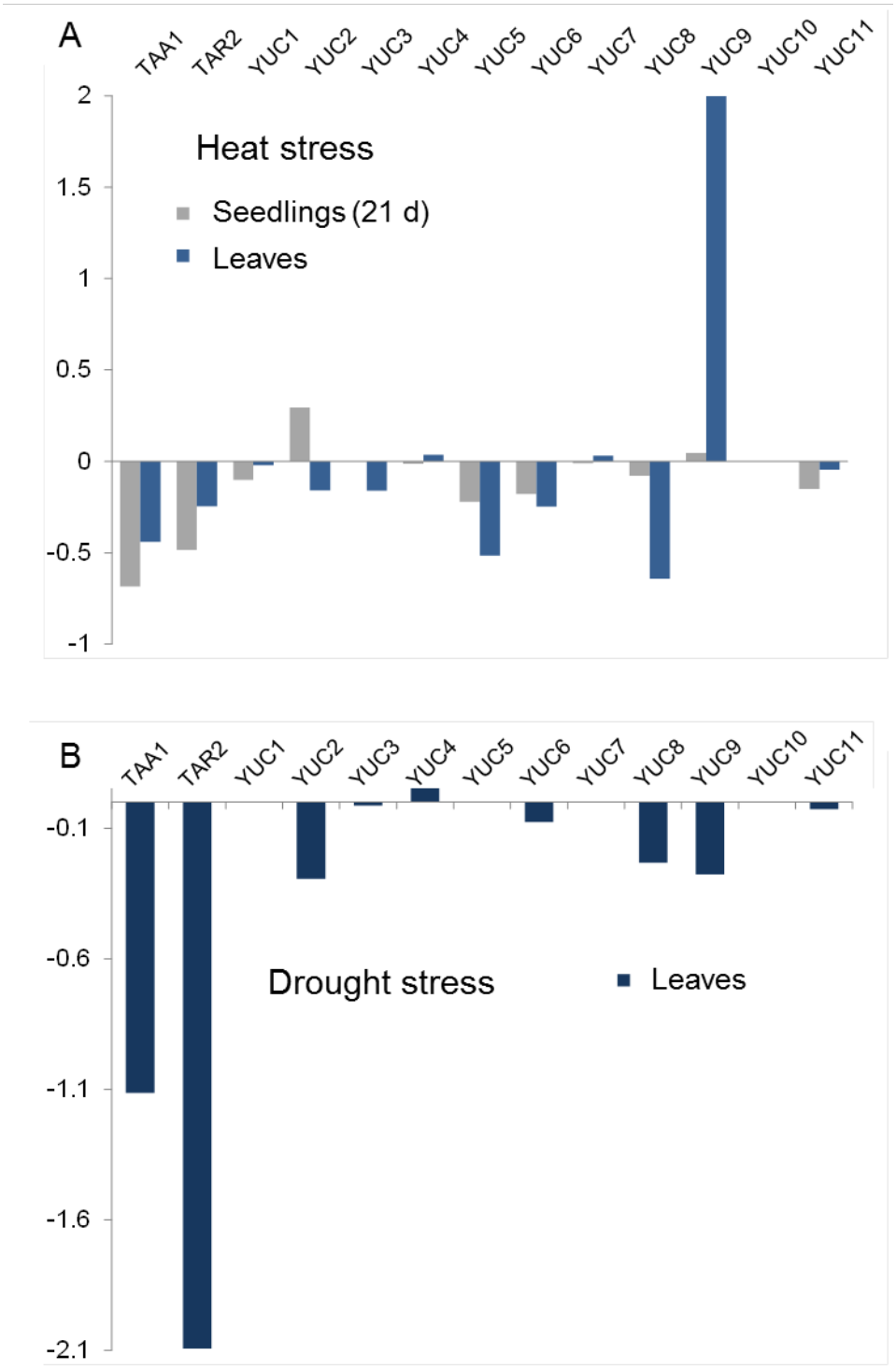

cytosol
ER
YUC1
YUC2
YUC4
YUC6
YUC11
Cytosol
YUC3
YUC5
YUC7
YUC8
YUC9
ER

Figure 4


A
TAA1
TAR2
YUC1
YUC2
YUC3
YUC4
YUC5
YUC6
YUC7
YUC8
YUC9
YUC10
YUC11
2
1.5
1
0.5
0
-0.5
-1
Heat stress
Seedlings (21 d)
Leaves
B
TAA1
TAR2
YUC1
YUC2
YUC3
YUC4
YUC5
YUC6
YUC7
YUC8
YUC9
YUC10
YUC11
-0.1
-0.6
-1.1
-1.6
-2.1
Drought stress
Leaves