

# Auxin response under osmotic stress

Victoria Naser<sup>1</sup> · Eilon Shani<sup>1</sup>

Received: 14 August 2015 / Accepted: 28 March 2016 / Published online: 6 April 2016  
© Springer Science+Business Media Dordrecht 2016

**Abstract** The phytohormone auxin (indole-3-acetic acid, IAA) is a small organic molecule that coordinates many of the key processes in plant development and adaptive growth. Plants regulate the auxin response pathways at multiple levels including biosynthesis, metabolism, transport and perception. One of the most striking aspects of plant plasticity is the modulation of development in response to changing growth environments. In this review, we explore recent findings correlating auxin response-dependent growth and development with osmotic stresses. Studies of water deficit, dehydration, salt, and other osmotic stresses point towards direct and indirect molecular perturbations in the auxin pathway. Osmotic stress stimuli modulate auxin responses by affecting auxin biosynthesis (*YUC*, *TAA1*), transport (*PIN*), perception (*TIR/AFB*, *Aux/IAA*), and inactivation/conjugation (*GH3*, *miR167*, *IAR3*) to coordinate growth and patterning. In turn, stress-modulated auxin gradients drive physiological and developmental mechanisms such as stomata aperture, aquaporin and lateral root positioning. We conclude by arguing that auxin-mediated growth inhibition under abiotic stress conditions is one of the developmental and physiological strategies to acclimate to the changing environment.

**Keywords** Auxin response · Auxin metabolism · Auxin biosynthesis · Auxin transport · Auxin perception · Osmotic stress · Drought stress · Abiotic stress · Hormone-stress crosstalk

---

✉ Eilon Shani  
eilonsh@post.tau.ac.il

<sup>1</sup> Department of Molecular Biology and Ecology of Plants, Tel Aviv University, 69978 Tel Aviv, Israel

## Introduction

Plant growth and development is highly plastic and dynamic. A single genotype possesses the capacity for a startling variety of morphologies and phenotypes, and within its lifetime, a single plant can adopt a variety of morphologies depending on the environmental conditions it is exposed to (de Jong and Leyser 2012). Climatic factors, such as extreme temperatures, drought, and saline soils are major abiotic environmental stressors that limit plant growth, reproductive success and ultimately yield. During the evolutionary shift from water to land, plants have had to acquire the tolerance to a number of osmotic stresses such as periods of soil and atmospheric water deficit (drought) and to high soil salinity (salinity), and they have thus acquired many strategies to survive exposure to osmotic stress (Chaves and Oliveira 2004). One such response to osmotic stress is an inhibition of growth, resulting in reduced stature but also reduced fertilization and ultimately reduced yield (Hsiao et al. 1976). The plant's growth response to stress conditions is characterized by a rapid and acute inhibition of growth termed "acute response", followed by recovery and acclimation to the new condition (Skirycz and Inzé 2010).

Virtually every aspect of plant growth and development, from embryogenesis to senescence, requires the plant hormone auxin (Vanderhoef and Dute 1981; Rayle and Cleland 1992; Benjamins and Scheres 2008). Processes governed by auxin in concert with other hormones include, among others, shoot and root meristem maintenance, tropic responses, leaf primordia and lateral root initiation, development of vascular tissues, control of apical dominance, and shoot and root elongation (Went and Thimann 1937; Abel and Theologis 1996). At the cellular level, auxin profoundly affects cell division, elongation and

differentiation to drive shaping forces of morphogenesis. Here, we review recent studies integrating osmotic stresses with the auxin pathway in plants. We dissect the auxin pathway into four molecular processes, auxin biosynthesis, transport, perception and cellular auxin response, and illustrate how osmotic stresses modulate these processes at the molecular level to modulate plant growth and development.

### Auxin biosynthesis in response to osmotic stress

#### *Auxin biosynthesis*

Auxins comprise an important group of hormones that are found in all land plants. Auxin biosynthesis is a tightly regulated process contributing to the control of auxin concentration in the cell and the formation of important auxin gradients in tissues. IAA (Indole-3-acetic acid) is the main auxin in higher plants (Bartel 1997), where a Trp- (tryptophan)-dependent two-step auxin biosynthesis pathway is proposed to be the major contributor to auxin production (Zhao et al. 2001; Stepanova et al. 2008; Tao et al. 2008; Yamada et al. 2009). In this pathway, Trp is first converted into IPA (indole-3-pyruvate) by the TAA1 (L-tryptophan pyruvate aminotransferase) family of aminotransferases, and then converted to IAA by the YUC (YUCCA) family of flavin monooxygenases (Mashiguchi et al. 2011; Won et al. 2011) (Fig. 1). Although additional Trp-dependent and Trp-independent IAA synthesis pathways are active in some species and in specific developmental processes [reviewed in (Tivendale et al. 2014; Wang et al. 2015)], the highly conserved TAA/YUC pathway seems to have a predominant role in plants (Zhao 2012; Gao and Zhao 2014).

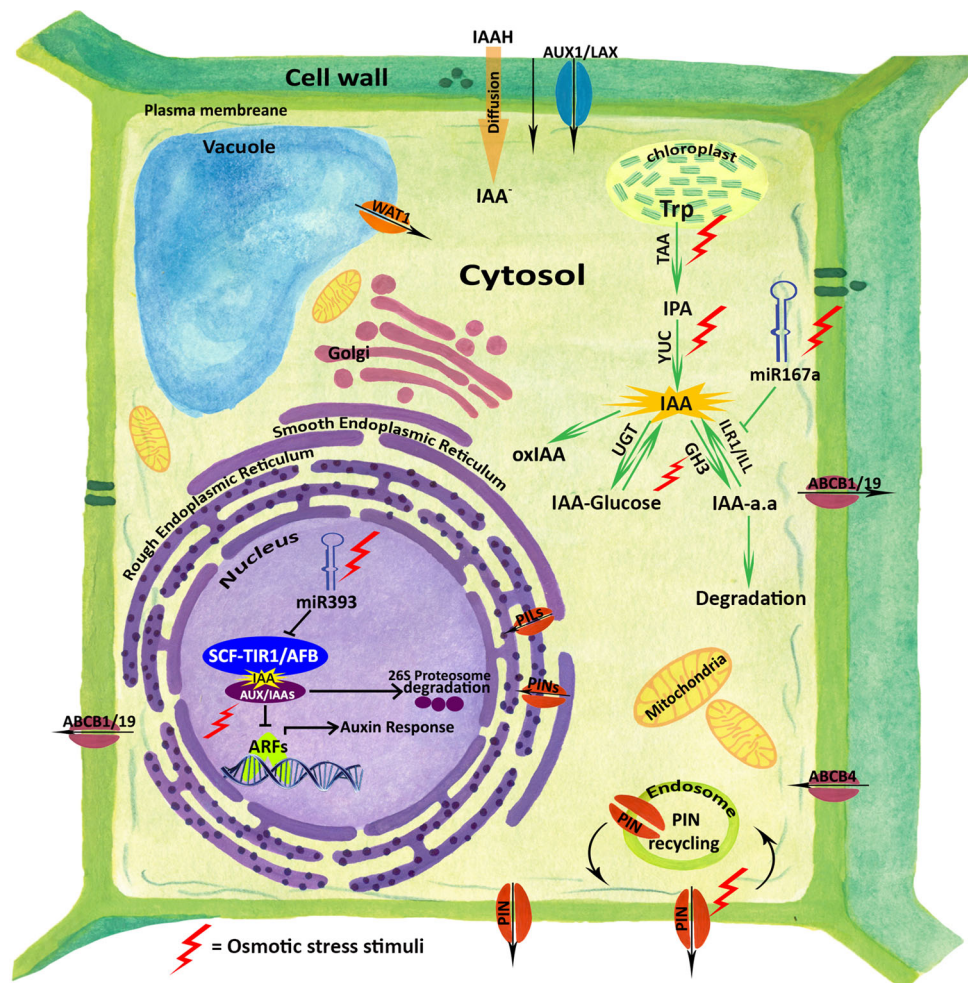
#### *Auxin biosynthesis and osmotic stress*

Direct measurements of endogenous IAA content in leaves and roots showed that IAA levels were significantly lower in plants growing in salt (NaCl) and water deficit conditions (Du et al. 2013; Liu et al. 2015). These results are consistent with a lower signal of the reporter *DR5* in root tips transferred to salty growth media, which is indicative of lower auxin response in this tissue (Wang et al. 2009; Zolla et al. 2009; Liu et al. 2015). Further molecular analyses revealed that plants exposed to water deficit conditions show a reduced expression of 6 out of 7 *YUC* auxin biosynthesis genes in rice (*Oryza sativa*) (Du et al. 2013). Inhibition of auxin biosynthesis is thus suggested to play a role in osmotic stress responses (Fig. 1). However, contradictory results show that several *YUC* genes in *Arabidopsis* are upregulated only hours after exposure to dehydration (2 h air drying on paper) (Lee et al. 2012). The

*Arabidopsis* activation-tagged mutant *yuc7-ID*, in which *YUC7* is up-regulated had higher total auxin levels compared to the WT but with similar free auxin levels. The plants exhibited phenotypes typical for an auxin overproducer, such as tall slender stems, enhanced apical dominance, curled narrow leaves, and were drought resistant (high water deficit survival rates) (Lee et al. 2012). In potato (*Solanum tuberosum*) and poplar (*Populus alba*, *P. glandulosa*), plants overexpressing *Arabidopsis YUC6* displayed phenotypes such as narrow downward-curved leaves and increased height, suggesting a high auxin content. These phenotypes were accompanied by enhanced drought tolerance (water withdrawal) due to reduced water loss (Im Kim et al. 2013; Ke et al. 2015). Consistent with an increased drought resistance obtained by auxin overproduction, *Arabidopsis iaaM-OX* transgenic lines, where higher IAA levels can be obtained by constitutively expressing *Agrobacterium tumefaciens tryptophan monooxygenase (iaaM)* gene, exhibited enhanced drought stress resistance (withdrawal of water for 21 days). At the same time, *yuc1/yuc2/yuc6* triple mutants with lower endogenous IAA level showed decreased stress resistance (Shi et al. 2014). Additional experiments suggest that auxin can promote drought stress resistance by modulating root architecture, ABA (abscisic acid)-responsive genes expression, and ROS (reactive oxygen species) metabolism (Shi et al. 2014).

The inconsistent results regarding auxin biosynthesis response to stress can arise from temporal stress response of minutes/hours versus days, thus pointing on a possible acute versus moderate stress response (Skirycz and Inzé 2010). In addition, the inconsistency may reflect the differential spatial response of different tissues and cell types to osmotic stress (Table 1). For example, specific *YUC* genes showed a differential stress response in different rice cultivars as well as an opposite response between water deficit and cold stresses (Du et al. 2013). An alternative explanation comes from a recent study that proposed that *YUC* genes confer drought tolerance independently of auxin biosynthesis by a novel thiol-reductase activity (Cha et al. 2015). These results suggest that the *YUC* proteins are playing a dual role of regulating plant development and conveying stress defence responses. Finally, the inconsistency may be a result of the dramatic effect of increased auxin biosynthesis on growth, which in turn may cause drought resistance/sensitivity. These secondary effects are discussed later.

The availability of water and minerals can be expected to have a major influence on root growth (Davies and Zhang 1991). Indeed, in experiments conducted in various growth media as well as in natural soil, it was found that roots growing along the surface of a wet medium produce more lateral roots on the side directly contacting the wet



**Fig. 1** A molecular model for osmotic stress integration into the cellular auxin pathway. *Auxin Biosynthesis, catabolism and conjugation*: Tryptophan is first converted into IPA by the TAA1 aminotransferase and IAA is subsequently produced from IPA by the YUC gene family. Both IAA biosynthetic steps are affected by osmotic stress. IAA could be further conjugated to other small molecules such as sugars by *UGT* and amino acids by the *GH3* families. The latter is reversible by the *ILR/ILL* amino acid hydrolases. Osmotic stress induces *GH3* genes and inhibits *miR167a* which targets *ILR3*. *Auxin Transport*: Auxin levels are modulated by controlling its cellular

transporter levels and intracellular localization. Osmotic stress affects PIN protein levels and localization by endocytosis and recycling. *Auxin Perception*: In the nucleus, auxin mediates the interaction between the auxin co-receptors Aux/IAA and SCF<sup>TIR1/AFB</sup>, leading to Aux/IAAs degradation and the release of ARFs from transcription inhibition. Osmotic stress effects the transcription level of several *Aux/IAAs* and triggers *miR393*, leading to *TIR1/AFB2* repression. Notably, the effect of the osmotic stress stimuli is not necessarily direct

surface compared to the air (Bao et al. 2014; Robbins and Dinneny 2015). In contrast, the air-exposed side has a higher abundance of root hairs. The term hydropatterning was coined to describe the patterning mechanism that positions lateral roots toward available water in the soil (Bao et al. 2014). Importantly, ABA signaling, known to inhibit the development of lateral roots, does not have a significant effect on hydropatterning. At the same time, water availability around the root promotes auxin biosynthesis and auxin accumulation in the root. *Arabidopsis* roots grown on media with a lower concentration of agar showed a significant increase in IAA concentration, increased auxin responses as suggested by an increased

*DR5-VENUS* signal (Ulmasov et al. 1997) and reduced levels of the *DII-VENUS* (Brunoud et al. 2012) auxin response reporters in the outer tissue layers of the root (Bao et al. 2014; Robbins and Dinneny 2015). These apparent increases in auxin could be specifically explained by an increased expression of the *TAA1* auxin biosynthesis gene, which showed significant expression differences between the water-contact and the air-exposed sides of the root. In line with the differential expression of *TAA1*, the *taal* mutant showed a significant reduction in hydropatterning that could be rescued by expressing *TAA1* specifically in the epidermis or throughout the root (Bao et al. 2014). Taken together, these results suggest that the local

**Table 1** Summary of osmotic stress integration into the auxin pathway

#	Species	Tissue	Stress treatment	Treatment results
Du et al. 2013	<i>Oryza sativa</i>	Leaves	Water deficit for 3 days after leaves start to curl	<i>YUC</i> genes suppressed, <i>GH3</i> genes upregulated, IAA levels decrease, altered expression of IAA signaling components ( <i>TIR2</i> , <i>Aux/IAA</i> , <i>ARF</i> )
Liu et al. 2015	<i>Arabidopsis thaliana</i>	Roots	Salinity: 100 mM NaCl, 2 days for root elongation and 24 h for <i>DR5:GFP</i> fluorescence	Inhibition of primary root elongation and meristem size, lower IAA levels, stabilization of IAA17 (reduction of IAA signaling)
Wang et al. 2009	<i>Arabidopsis thaliana</i>	Roots	Salinity: 25, 100, 150, 200 mM NaCl	PR elongation increase and LR development inhibition under low salinity, PR inhibition and LR development induction under high salinity
Zolla et al. 2009	<i>Arabidopsis thaliana</i>	Roots	Salinity/osmotic stress: 50 mM NaCl/100 mM mannitol	Osmotic stress inhibits primary and lateral root elongation. NaCl stimulates LR proliferation while mannitol does not
Lee et al. 2012	<i>Arabidopsis thaliana</i>	Whole plants	Desiccation, 2 h for gene expression; water withheld for 20 days for water deficit resistance	<i>YUC7</i> , 9, 10, 11 genes were induced; <i>yuc7-ID</i> mutant was tolerant to water deficit
Im Kim et al. 2013	<i>Solanum tuberosum</i>	Whole plants	Water deficit: water withheld for 18 days	<i>AtYUC6</i> overexpressing transgenic potato plants were drought tolerant
Ke et al. 2015	<i>Populus alba</i> × <i>P. glandulosa</i>	Whole plants	Water deficit for 6 days	<i>AtYUC6</i> overexpressing plants lost less water and were drought tolerant
Shi et al. 2014	<i>Arabidopsis thaliana</i>	Whole plants	Water deficit: water withheld for 21 days	<i>iaaM-OX</i> presented improved water drought tolerance, <i>yuc1/2/6</i> triple mutants displayed water deficit sensitivity
Bao et al. 2014	<i>Arabidopsis</i> , <i>Oryza sativa</i> , <i>Zea mays</i>	Roots	Differential water availability—one side of the primary root exposed to air	Water availability in the contact side promotes auxin transport, accumulation, signaling, and response leading to LR development
Park et al. 2007	<i>Arabidopsis thaliana</i>	Whole plants	Water deficit for 14 days/salinity: 200 mM NaCl for 3 days	Overexpression of a <i>GH3</i> gene leads to lower IAA levels, growth retardation and tolerance to abiotic stresses
Zhang et al. 2009	<i>Oryza sativa</i>	Whole plants	Water deficit: withholding water for 7 days. Desiccation: root exposure to air, 24 h for gene expression. Salinity: 200 mM NaCl for 24 h	<i>GH3</i> expression was induced and free-IAA levels reduced by water deficit and salinity. Gain of function in a <i>GH3</i> gene leads to lower free-IAA levels decreased water loss and enhanced water deficit tolerance
Wang et al. 2010	<i>Sorghum bicolor</i>	Whole plants	Water deficit for 7 days and 150 mM NaCl for 7 days	Water deficit and salinity lead to the induction of <i>GH3-1</i> , 2, 4, 5, 12, 13, <i>SbIAA1</i> , <i>SbGH3-13</i> , and <i>SbLBD32</i>
Du et al. 2012	<i>Oryza sativa</i>	Whole plants	Water withdrawal for 4 days	<i>OsGH3-2</i> rapidly induced. Overexpression of <i>osGH3</i> lead to lower IAA levels, faster water loss and hypersensitivity to water deficit
Feng et al. 2015	<i>Zea mays</i>	Whole plants	Salinity: 150 mM NaCl for 4 days, osmotic stress: 20 % mannitol for 4 days	<i>ZmGH3</i> genes were largely induced by salinity. Root IAA content was significantly decreased in both NaCl and Mannitol treatments
Kinoshita et al. 2012	<i>Arabidopsis thaliana</i>	Whole plants	Water deficit: withholding water for 17 days	Reduction in <i>miR167</i> accumulation, a two fold increase in <i>IAR3</i> mRNA levels
West et al. 2004	<i>Arabidopsis thaliana</i>	Roots	Salinity: 0.3–1 % NaCl for 7 days	Salinity leads to a reduction in root stem meristem size and an inhibition of root growth
Galvan-Ampudia and Testerink 2011	<i>Arabidopsis thaliana</i>	Roots	Salinity: replacing the lower half of the agar medium with 200 mM NaCl for 6 days	Salinity leads to a LR development inhibition

**Table 1** continued

#	Species	Tissue	Stress treatment	Treatment results
Zhao et al. 2011	<i>Arabidopsis thaliana</i>	Roots	30 mM NaCl for 10 days; 25 mM mannitol for 10 days	<i>SOS3</i> affects auxin basipetal transport and induction of LR under low salt stress
Galvan-Ampudia et al. 2013	<i>Arabidopsis</i> , <i>Sorghum</i> , <i>S. lycopersicum</i>	Roots	Salinity diagonal gradients of NaCl (150–300 mM), for 24, 48, 72 h	Increased auxin levels at the side of the root opposite to the higher salinity leading to the shaping force of halotropism
Dinneny et al. 2008	<i>Arabidopsis thaliana</i>	Roots	Salinity: 0–250 mM NaCl for 4–6 days halotropism assays. 150 mM NaCl for 2–8 h gene expression	Salt modulates root growth direction by reducing the gravity response
Nakayama et al. 2012	<i>Solanum lycopersicum</i>	Shoot	Osmotic stress: 0–400 mM mannitol for 2 h	Hypoosmolarity (0 mM) increased PIN1 density on the PM, while hyperosmolarity (200–400 mM) decreased it
Zwiewka et al. 2015	<i>Arabidopsis thaliana</i>	Root meristem	Salinity: 100 mM NaCl or 75 mM sorbitol	Hyperosmotic conditions activate PIN1 and PIN2 internalization (endocytosis) through the clathrin-mediated pathway
Song et al. 2009	<i>Oryza sativa</i>	Leaves	Desiccation: air drying on paper for 0–12 h. Salinity: 200 mM NaCl for 0–12 h. osmotic stress: 0–150 mM mannitol for 10 days	Several <i>IAA</i> and <i>ARF</i> genes were upregulated by at least one of the treatments, with a great overlap between the genes responsive to water deficit and salt stress
Jung et al. 2015	<i>Oryza sativa</i>	Whole plants	Desiccation: air drying for 2 h at 28 °C. Salinity: 400 mM NaCl 2 h at 28 °C. Water deficit for 5 days	<i>OsIAA6</i> was induced under water deficit and salt stress. Overexpression of <i>OsIAA6</i> in rice improved tolerance to drought
Iglesias et al. 2010	<i>Arabidopsis thaliana</i>	Whole plants	Salinity: 50–250 mM NaCl for 3–7 days	<i>tir1afb2</i> double mutant is tolerant to salt stress
Chen et al. 2014	<i>Arabidopsis thaliana</i>	Whole plants	Salinity: 0, 100 or 150 mM NaCl for 9 days	<i>miR393</i> is induced under salt stress. Salt stress tolerance is enhanced in <i>mTIR1</i> -overexpressing plants
Iglesias et al. 2014	<i>Arabidopsis thaliana</i>	Roots	Salinity: 50–200 mM NaCl 4 h for GUS; 75 mM NaCl 5–7 days for LR assays	Salinity induces <i>miR393</i> expression which in turn reduces <i>TIR1/AFB</i> levels and LR and MR elongation inhibition
Park et al. 2011	<i>Arabidopsis thaliana</i>	Whole plants	Salinity: 50–150 mM NaCl, 4 days for germination assays and 14 days for seedling growth assays	Salinity upregulates <i>NTM2</i> which induces <i>IAA30</i> and stress mediated germination inhibition

differential water availability promotes auxin accumulation and auxin response.

### Auxin catabolism and conjugation in response to osmotic stress

#### *Auxin catabolism and conjugation*

Auxin catabolism and conjugation may determine the actual level of active IAA in a given cell and consequently play an important role in many developmental processes. Importantly in higher plants, the bulk of IAA is stored in a conjugated form, providing free active IAA once needed in various developmental processes (Ludwig-Müller 2011). IAA can be converted to ester conjugates with sugars via UGTs (UDP-glucose transferases) or to amide conjugates with amino acids by IAA amino acid conjugate synthetases of the *GH3* (*Gretchen Hagen3*) family (Staswick et al. 2005; Ludwig-Müller 2011) (Fig. 1). Most IAA amino acid conjugates such as IAA-Ala (alanine), IAA-Leu (leucine),

IAA-Phe (phenylalanine) can then again be hydrolyzed by the ILR1/ILL (IAA-LEUCINE RESISTANT1/IAA-LEUCINE RESISTANT1 (ILR1)-LIKE) proteins to release free IAA (Bartel and Fink 1995; Woodward and Bartel 2005) whereas amino acid conjugates such as IAA-Asp (aspartic acid) and IAA-Glu (glutamic acid) are thought to be precursors of an IAA degradation pathway that cannot be reconverted. Local auxin levels could be buffered by the irreversible oxydization of IAA, leading to the inactive product oxIAA (Peer et al. 2013).

#### *Auxin catabolism and conjugation under osmotic stress*

*GH3.5* is rapidly induced by various stress conditions. The dominant *gh3.5* allele of the gene *WES1* exhibits several auxin-deficient traits, including growth retardation and altered leaf shape, and it shows resistance to biotic (*P. syringae* infections) and abiotic stresses (withdrawal of water and salinity) (Park et al. 2007). The results suggest that auxin homeostasis plays an important role in mediating

growth in response to stress. It is therefore suggested that coordinating auxin-mediated growth control is an important mechanism to cope with the changing stressed environment.

In rice shoots, the *IAA-amidosynthetase GH3.13* (rice *TLD1*, *increased number of tillers, enlarged leaf angles, and dwarfism*) is induced by auxin and water deficit stress (Hagen and Guilfoyle 1985; Staswick et al. 2005). Furthermore, in sorghum, maize, and rice, altered expression of *GH3* family gene members was shown to occur in response to several abiotic stresses (Zhang et al. 2009; Wang et al. 2010; Du et al. 2012; Feng et al. 2015) (Fig. 1). The activation of *TLD1* reduced local *DR5:GUS* signal, resulting in subsequent alterations in plant architecture and enhanced drought tolerance (Zhang et al. 2009; Liu et al. 2014). *OsGH3-2* is rapidly induced by water deficit stress in rice. Interestingly, overexpression of *OsGH3-2* results in drought hypersensitivity (low water deficit survival rate) and increased resistance to cold stress (Du et al. 2012). Altogether, the molecular data indicate that IAA conjugation plays an important role in plant stress response, albeit some molecular and physiological inconsistency is apparent. Spatiotemporal stress response variation as well as secondary growth affects (discussed later in the conclusions) may explain this inconsistency (Table 1).

The active and regulated process of IAA hydrolysis leads to a rapid release of free IAA from IAA sugar or amino acid conjugates. IAR3 hydrolyzes an inactive form of auxin (IAA-alanine) and releases bioactive IAA (Bartel and Fink 1995; Woodward and Bartel 2005). Recent work shows that high osmotic stress inhibits *miR167a* levels thereby increasing the mRNA levels of its direct target *IAR3* (Fig. 1). *iar3* mutants show reduced levels of free IAA and display reduced osmotic stress-induced root architecture to achieve partial drought tolerance (Kinoshita et al. 2012; Liu et al. 2014). Further studies are needed to explore the molecular mechanisms of auxin homeostasis during plant acclimation to water deficit. For example, it will be important to understand whether subcellular compartments, such as vacuoles (Löffke et al. 2015), regulate the spatial distribution of conjugated and free auxin in response to stress.

### Auxin transport in response to osmotic stress

#### Auxin transport

Auxin is polarly transported in plants by cell-to-cell trafficking through specific transporter proteins. Their combined activity results in the formation of IAA gradients and local maxima that account for many important developmental processes such as embryo development, lateral root and leaf primordia initiation (Jacobs and Rubery 1988; Reinhardt et al. 2003; Zažímalová et al. 2010). Several auxin transporters act in concert to control polar auxin transport, local

auxin concentrations and auxin homeostasis: PIN (PIN-FORMED) proteins are auxin efflux carriers and their polar distribution in many cells of the plant promotes directional auxin efflux out of the cells (Gälweiler et al. 1998; Wiśniewska et al. 2006; Petrášek and Friml 2009). PGP/ABCB1,19 (P-glycoproteins/ATP-binding cassette class B proteins) are uniformly distributed in the plasma membranes and control non-polar auxin efflux (Geisler and Murphy 2006; Peer et al. 2011). Once exported, auxin can enter cells by diffusion when protonated or through the AUX1/LAX (AUXIN TRANSPORTER CARRIER1/AUXIN TRANSPORTER-LIKE PROTEINS) influx carriers (Swarup et al. 2001; Kramer and Bennett 2006) (Fig. 1). Recent studies have identified two additional auxin transporters, namely PILS (PIN-LIKES) and WAT1 (WALLS ARE THIN1), located on intracellular membrane compartments, the ER, and vacuoles, respectively (Barbez et al. 2012; Ranocha et al. 2013).

#### Auxin transport and osmotic stress

Salt stress inhibits primary root growth through a decrease in cell division and elongation (West et al. 2004). In addition, salt stress inhibits lateral root formation (Wang et al. 2009; Galvan-Ampudia and Testerink 2011). The inhibition of root growth by salt stress correlates with reduced auxin responses as indicated by a reduced *DR5* signal in the root tip and in potential lateral root initiation sites (Wang et al. 2009; Zolla et al. 2009; Liu et al. 2015). Interestingly, mild salinity stimulates both initiation and emergence of lateral roots (Zolla et al. 2009; Zhao et al. 2011). Since auxin transport is an important level of regulation in auxin spatiotemporal homeostasis, it is interesting to understand whether osmotic stress affects this process. This can be approached in two different ways by choosing auxins that are independent from the auxin efflux and influx proteins or by manipulating the abundance of the auxin transporter proteins. Unlike IAA, 2,4-D (2,4-dichlorophenoxyacetic acid), is not efficiently effluxed from cells. Interestingly, 2,4-D treatments have a strong effect on the position of lateral roots toward available water in the soil (hydropatterning). Furthermore, manipulations of PIN proteins, e.g. by *PIN1* overexpression or in the *pin2/3/7* triple mutant, resulted in a disrupted hydropatterning, indicating that PIN-mediated auxin efflux is required for hydropatterning (Bao et al. 2014). Furthermore, *PIN3* translational reporter (*ProPIN3:PIN3:GFP*) had a specific enrichment in cortex and endodermal cells (ground tissue) overlying early-stage lateral-root primordia on the contact side with an agar medium in comparison to the air side. These results suggest that PIN3 plays an important role in mediating the hydraulic conductivity driving hydropatterning (Bao et al. 2014).

The mechanism that allows directional growth of roots away from regions of high salt concentrations has been coined halotropism (Rosquete and Kleine-Vehn 2013). Salt stress modulates the direction of root growth by reducing the gravity response (Dinneney et al. 2008; Sun et al. 2008). Since auxin has a major role in mediating the root gravitropic response, recent studies have searched for the molecular mechanism linking halotropism and auxin transport. Galvan-Ampudia et al. show that the auxin response reporter *DII-VENUS* is reduced in response to a directional gradient salt treatment, thus indicating a possible increase in auxin levels at the side of the root that is most distant to the higher salinity (Galvan-Ampudia et al. 2013). Exogenous auxin treatment abolished the root's halotropic response to salt (Galvan-Ampudia et al. 2013). A recent study shows that hydrotropism, which is the directional growth of roots toward regions of highest water potential does not require auxin redistribution (Shkolnik et al. 2016), suggesting for an exceptional auxin independent tropism.

Several recent reports suggest that osmotic treatments impair maintenance of PIN polarity, but the individual results contradict each other with regard to the PINs involved and the type of stress (Nakayama et al. 2012; Galvan-Ampudia et al. 2013; Zwiewka et al. 2015) (Table 1). With regard to root growth orientation, the role of PIN2 is particularly interesting since PIN2 also regulates root gravitropism (Abas et al. 2006). Salt gradients (anionic osmotic stress) but not mannitol (non-anionic osmotic stress) induce the internalization of PIN2 specifically at the side of the root facing a high salt concentration. Thus, halotropism response correlates with PIN2 protein localization (Sun et al. 2008). No effect of salt treatment was found on the subcellular localization or internalization of the other auxin transporters involved in tropic response, suggesting that PIN2 specifically mediates roots halotropic response to salt by affecting auxin flow (Galvan-Ampudia et al. 2013). Experiments testing PIN1 localization in tomato shoot apical meristems showed that osmotic stress affects PIN1 protein level and intracellular localization (Nakayama et al. 2012). Salt stress represses the expression of PIN genes, resulting in short root meristems (Liu et al. 2015). Consistently, osmotic treatments of NaCl and manitol to root meristem cells immediately increased the internalization of PIN1 and PIN2 and decreased the recycling of internalized PINs to the plasma membrane (Zwiewka et al. 2015), thus supporting the finding that acute hyperosmotic stress enhances PIN endocytosis (Nakayama et al. 2012; Galvan-Ampudia et al. 2013; Zwiewka et al. 2015). Further work should try to address why halotropism is specific to salt stress while the effects on PIN maintenance and polarity are promoted by several different osmotic conditions.

## Auxin perception in response to osmotic stress

### Auxin perception

Auxin is perceived by a co-receptor complex consisting of an F-box protein from the TIR1/AFB (TRANSPORT INHIBITOR RESPONSE 1/AUXIN SIGNALING F-BOX PROTEIN) family and a member of the Aux/IAA (AUXIN/INDOLE-3-ACETIC ACID) family of transcriptional repressors (Wang and Estelle 2014). Auxin acts as a “molecular glue” to promote the interaction between TIR1/AFBs and Aux/IAAs, leading to ubiquitination and 26S proteasome-mediated degradation of the Aux/IAAs. Aux/IAA degradation de-represses the transcription responses of the Aux/IAA-interacting ARF (AUXIN RESPONSE FACTOR) proteins (Bargmann and Estelle 2014; Salehin et al. 2015). Thus, auxin triggers a vast response network by increasing the affinity between the TIR1/AFB and Aux/IAA coreceptor families, which in turn modulates ARF-dependent molecular outputs (Fig. 1). Importantly, all three protein families involved in auxin response are comprised of numerous family members (29 Aux/IAA, 23 ARF, and 6 TIR1/AFB members in *Arabidopsis*) and the formation of a large heterogenous number of protein complexes can be envisioned to differentially affect auxin responses (Wang and Estelle 2014). Recent structural studies suggested that formation of Aux/IAA multimers is required for efficient repression of auxin signaling (Korasick et al. 2014; Nanao et al. 2014; Guilfoyle 2015; Korasick et al. 2015), while ARF proteins homodimerize to generate cooperative DNA binding (Boer et al. 2014; Korasick et al. 2015).

### Auxin perception and osmotic stress

In rice, water deficit and salt stress were shown to modulate auxin responses (Du et al. 2013). Microarray gene expression under stress conditions revealed the differential regulation of several members of the *GH3*, *Aux/IAA*, *SAUR* (*Small Auxin Upregulated RNA*), and *ARF* families, which all represent typically auxin-regulated genes (Song et al. 2009; Du et al. 2013). Specifically, several members of the *Aux/IAAs* have shown a response to water deficit stress (Song et al. 2009) (Fig. 1). Overexpression of the water deficit -induced *Aux/IAA6* improved drought tolerance in rice (Jung et al. 2015). As *Aux/IAA6* overexpression affected the expression of auxin biosynthesis genes, it is difficult to interpret whether the drought tolerance reflects the outcome of a direct auxin signaling response or a secondary response that results in altered auxin levels by modulating auxin biosynthesis gene expression (Jung et al. 2015). In agreement with a reduction of auxin responses after salt stress, it was found in *Arabidopsis* that the

stabilization of *IAA17* leads to a salt-mediated inhibition of root meristem growth (Liu et al. 2015).

The auxin resistant double mutant *tir1 afb2* is more tolerant to salt and oxidative stress compared to wild-type (Iglesias et al. 2010), suggesting that down-regulation of auxin signaling might mediate plant acclimation to stress. Salinity triggers *miR393* expression (Chen et al. 2014), which targets the *TIR1* and *AFB2* auxin receptors to mediate the repression of lateral root development during salinity (Iglesias et al. 2014) (Fig. 1). Furthermore, overexpression of a *miR393-resistant TIR1* gene (*mTIR1*) showed increased tolerance to salt stress as well as reduced hydrogen peroxide ( $H_2O_2$ ) and superoxide anion ( $O_2^-$ ) levels (Iglesias et al. 2010; Chen et al. 2014). These findings suggest that osmotic stress might suppress *TIR1/AFB*-mediated auxin signaling in the root, thereby incorporating stress signals into growth inhibition response under stress conditions.

The studies described above illustrate that osmotic stress rapidly affects the auxin response, and that this response may lead to drought tolerance. Several other studies suggest however different physiological mechanisms correlating auxin signaling and drought tolerance. Three recent papers have shown that auxin is an important regulator of stomatal development. In response to water deficit stress, plants trigger stomatal closure to reduce water loss (Schroeder et al. 2001). Perturbations of polar auxin transport, resulting either from treatment with polar auxin transport inhibitors or from mutations in multiple genes of the *PIN* family of auxin efflux carriers, cause stomatal clustering (Le et al. 2014). Accordingly, application of exogenous auxin reduces the stomata development, whereas mutants disrupted in the *taa1* auxin biosynthesis pathway display increased stomatal clustering (Balcerowicz et al. 2014; Zhang et al. 2014). Auxin response reporter activities drop at specific stages of the meristemoid differentiation, suggesting that auxin depletion might be required for the switch from unequal to equal division (Le et al. 2014). Loss-of-function mutations in the functionally redundant *TIR1* and *AFB1* auxin receptor genes, or the *ARF* gene *MP* (*MONOPTEROS*) as well as a gain-of-function mutations in *AUX/IAA12* (*BDL*) and *AUX/IAA17* (*AXR3*) result in strong stomatal clustering (Balcerowicz and Hoecker 2014; Balcerowicz et al. 2014; Zhang et al. 2014). Altogether, these results identify auxin as an important repressor of stomata differentiation (Balcerowicz and Hoecker 2014). Future work should test how moderate osmotic stress integrates into the auxin-signaling pathway to drive long-term stomata developmental acclimation.

In addition to the effects of auxin on stomata and drought tolerance, auxin was also shown to coordinate specific aquaporin function. Auxin promotes lateral root development by regulating the distribution of aquaporin-dependent root water transport (Péret et al. 2012). During lateral root

initiation, auxin reduces the expression of several *AtPIP* and *AtTIP* genes, including *AtPIP2;1*, which is excluded from the lateral root primordia but maintained at their base. Thus, auxin mediates cell and tissue hydraulic properties during lateral root development (Péret et al. 2012).

While stomata development and aquaporin position are straightforward in terms of drought tolerance mechanisms, a recent study suggests that auxin signaling affects seed germination in salty environment. *NTM2* (*NAC* transcription factor) integrates auxin and salt signals during seed germination (Park et al. 2011). Seed germination is delayed by auxin under high salinity in a *NTM2* dependent manner, possibly by a rapid induction of *IAA30* in response to salt stress (Park et al. 2011). It will be interesting to explore whether the auxin mediated germination response regulates growth and cell division processes that are modulated by stress, or rather the process involves other known stress and germination hormones such as GA (gibberellin) and ABA.

## Conclusions

Environmental stress potentially disrupts cellular structures and impairs key physiological functions. Water deficit, salinity and dehydration stresses impose an osmotic stress that restricts water uptake. As a consequence, membranes may become disorganized, proteins may denature, chloroplasts may lose their photosynthetic activity and reactive oxygen species (ROS) are often produced leading to oxidative damage, all contributing to growth perturbation (Zhu 2002). In addition, recent studies show that growth retardation is a direct response to osmotic stress (Skirycz and Inzé 2010). Why do plants limit their growth in response to stress as a direct and active process? Elegant studies from the 1970's show that plants with smaller cell size are more tolerant of low water potential and water deficit (Cutler et al. 1977; Steudle et al. 1977; Bradford and Hsiao 1982). Therefore, plants that rapidly respond to the changing stressed environment and limit their growth, in particular their cell size, have better physical parameters to survive water deficit periods. This raises the possibility that plants utilize the auxin response as a direct mechanism to rapidly regulate growth under osmotic stress. Several studies outlined in this review support this hypothesis. However, it is difficult to exclude the possibility that auxin manipulation, which in many cases drives direct growth inhibition and alters many morphological parameters, generates drought tolerance indirectly. A large number of studies showing overexpression phenotypes, either related to auxin or not, which display growth defects, claim for drought resistance/sensitivity gene function, while it is difficult to separate between the direct effects of the respective genes and secondary growth defects resulting



from gain-of-function manipulation leading to drought resistance or sensitivity. Both, auxin and osmotic stress signaling pathways are complex and highly redundant, limiting the effectiveness of forward genetics studies (Bouché and Bouchez 2001; Takeda and Matsuoka 2008; Mittler and Blumwald 2010). Further precise and creative work is needed to explore the direct crosstalk between osmotic stress and auxin response.

So far, the studies exploring the stress and auxin pathway lack the spatiotemporal resolution. Temporally, both stress and auxin pathways are rapid and transient pulses that might drive growth processes days and weeks later. Most experiments exploring the transcriptional response to osmotic stress were carried in a minute and hour time-scales, and therefore primarily give insight on the acute osmotic response. However, most physiological growth experiments are carried out on a time scale of days and weeks, therefore primarily representing the adaptive growth stage. In order to advance our understating of plant acclimation to the stressed environment, the scientific community will need to generate novel approaches and tools to monitor and integrate the continuous acute and adaptive growth processes. Spatially, both stress and auxin responses are often tissue and cell-type specific (Dinneny et al. 2008; Bargmann et al. 2013). The studies carried so far in the intersection of auxin and stress responses were of a general nature of the integrative plant. Recent elegant work showed that the endodermis is an important signaling center during the regulation of lateral root growth in high saline conditions (Duan et al. 2013; Geng et al. 2013; Duan et al. 2015). Corresponding studies are necessary to fill the spatial gaps in the auxin/stress cell-type specific crosstalk.

While this review has focused on the intersection between osmotic stress conditions and its effect on the auxin response at the molecular, physiological and developmental aspects, it is clear that these processes also require a broad set of additional proteins and regulators. The plant hormones ABA (Zwack and Rashotte 2015), CK (Peleg et al. 2011; Zwack and Rashotte 2015), ethylene (Morgan and Drew 1997; Alonso et al. 1999) and gibberellin (Achard et al. 2006, 2008; Nir et al. 2014) are all known to mediate the drought response in plants (review in (Peleg and Blumwald 2011)). The knowledge about the crosstalk between auxin and these hormones (Stepanova et al. 2007; Weiss and Ori 2007; Moubayidin et al. 2009; Shkolnik-Inbar and Bar-Zvi 2010) further supports the link between osmotic stress and auxin. Future studies should reveal the direct molecular mechanisms behind auxin regulation of stress-induced growth patterning in plants.

**Acknowledgments** We thank Claus Schwechheimer (TUM), Doron Shkolnik-Inbar (TAU) and Mark Estelle (UCSD) for critically reading the article and for helpful suggestions. Research on related topics in

the Shani lab is supported by grants from the Israel Science Foundation (1832/14) and (2158/14), GIF, the German-Israeli Foundation for Scientific Research and Development (I-236-203.17-2014), and the Human Frontier Science Program (HFSP-RGY0075/2015).

**Author contribution** Victoria Naser and Eilon Shani wrote the manuscript.

## References

- Abas L, Benjamins R, Malenica N, Paciorek T, Wiśniewska J, Moulinier-Anzola JC, Sieberer T, Friml J, Luschnig C (2006) Intracellular trafficking and proteolysis of the Arabidopsis auxin-efflux facilitator PIN2 are involved in root gravitropism. *Nat Cell Biol* 8:249–256
- Abel S, Theologis A (1996) Early genes and auxin action. *Plant Physiol* 111:9
- Achard P, Cheng H, De Grauwe L, Decat J, Schoutteten H, Moritz T, Van Der Straeten D, Peng J, Harberd NP (2006) Integration of plant responses to environmentally activated phytohormonal signals. *Science* 311:91–94
- Achard P, Gong F, Cheminant S, Alioua M, Hedden P, Genschik P (2008) The cold-inducible CBF1 factor-dependent signaling pathway modulates the accumulation of the growth-repressing DELLA proteins via its effect on gibberellin metabolism. *Plant Cell* 20:2117–2129
- Alonso JM, Hirayama T, Roman G, Nourizadeh S, Ecker JR (1999) EIN2, a bifunctional transducer of ethylene and stress responses in Arabidopsis. *Science* 284:2148–2152
- Balcerowicz M, Hoecker U (2014) Auxin—a novel regulator of stomata differentiation. *Trends Plant Sci* 19:747–749
- Balcerowicz M, Ranjan A, Rupprecht L, Fiene G, Hoecker U (2014) Auxin represses stomatal development in dark-grown seedlings via Aux/IAA proteins. *Development* 141:3165–3176
- Bao Y, Aggarwal P, Robbins NE 2nd, Sturrock CJ, Thompson MC, Tan HQ, Tham C, Duan L, Rodriguez PL, Vernoux T, Mooney SJ, Bennett MJ, Dinneny JR (2014) Plant roots use a patterning mechanism to position lateral root branches toward available water. *Proc Natl Acad Sci USA* 111:9319–9324
- Barbez E, Kubeš M, Rolčik J, Béziat C, Pěnčík A, Wang B, Rosquete MR, Zhu J, Dobrev PI, Lee Y, Zažímalová E, Petrášek J, Geisler M, Friml J, Kleine-Vehn J (2012) A novel putative auxin carrier family regulates intracellular auxin homeostasis in plants. *Nature* 485:119–122
- Bargmann BO, Estelle M (2014) Auxin perception: in the IAA of the beholder. *Physiol Plant* 151:52–61
- Bargmann BO, Vanneste S, Krouk G, Nawy T, Efroni I, Shani E, Choe G, Friml J, Bergmann DC, Estelle M, Birnbaum KD (2013) A map of cell type-specific auxin responses. *Mol Syst Biol* 9:688
- Bartel B (1997) Auxin biosynthesis. *Annu Rev Plant Biol* 48:51–66
- Bartel B, Fink GR (1995) ILR1, an amidohydrolase that releases active indole-3-acetic acid from conjugates. *Science* 268:1745
- Benjamins R, Scheres B (2008) Auxin: the looping star in plant development. *Annu Rev Plant Biol* 59:443–465
- Boer DR, Freire-Rios A, van den Berg WA, Saaki T, Manfield IW, Kepinski S, López-Vidriero I, Franco-Zorrilla JM, de Vries SC, Solano R, Weijers D, Coll M (2014) Structural basis for DNA binding specificity by the auxin-dependent ARF transcription factors. *Cell* 156:577–589
- Bouché N, Bouchez D (2001) Arabidopsis gene knockout: phenotypes wanted. *Curr Opin Plant Biol* 4:111–117
- Bradford K, Hsiao T (1982) Physiological responses to moderate water stress. In: *Physiological plant ecology II*. Springer, Berlin, pp 263–324

- Brunoud G, Wells DM, Oliva M, Larrieu A, Mirabet V, Burrow AH, Beeckman T, Kepinski S, Traas J, Bennett MJ, Vernoux T (2012) A novel sensor to map auxin response and distribution at high spatio-temporal resolution. *Nature* 482:103–106
- Cha JY, Kim WY, Kang SB, Kim JI, Baek D, Jung IJ, Kim MR, Li N, Kim HJ, Nakajima M, Asami T, Sabir JS, Park HC, Lee SY, Bohnert HJ, Bressan RA, Pardo JM, Yun DJ (2015) A novel thiol-reductase activity of Arabidopsis YUC6 confers drought tolerance independently of auxin biosynthesis. *Nat Commun*. doi:10.1038/ncomms9041
- Chaves M, Oliveira M (2004) Mechanisms underlying plant resilience to water deficits: prospects for water-saving agriculture. *J Exp Bot* 55:2365–2384
- Chen Z, Hu L, Han N, Hu J, Yang Y, Xiang T, Zhang X, Wang L (2014) Overexpression of a miR393-resistant form of transport inhibitor response protein 1 (mTIR1) enhances salt tolerance by increased osmoregulation and Na<sup>+</sup> exclusion in Arabidopsis thaliana. *Plant Cell Physiol* 56:73–83
- Cutler J, Rains D, Loomis R (1977) The importance of cell size in the water relations of plants. *Physiol Plant* 40:255–260
- Davies WJ, Zhang J (1991) Root signals and the regulation of growth and development of plants in drying soil. *Annu Rev Plant Biol* 42:55–76
- de Jong M, Leyser O (2012) Developmental plasticity in plants. In: Cold spring harbor symposia on quantitative biology. Cold Spring Harbor Laboratory Press, pp 63–73
- Dinneny JR, Long TA, Wang JY, Jung JW, Mace D, Pointer S, Barron C, Brady SM, Schiefelbein J, Benfey PN (2008) Cell identity mediates the response of Arabidopsis roots to abiotic stress. *Science* 320:942–945
- Du H, Wu N, Fu J, Wang S, Li X, Xiao J, Xiong L (2012) A GH3 family member, OsGH3-2, modulates auxin and abscisic acid levels and differentially affects drought and cold tolerance in rice. *J Exp Bot* 63:6467–6480
- Du H, Liu H, Xiong L (2013) Endogenous auxin and jasmonic acid levels are differentially modulated by abiotic stresses in rice. *Front Plant Sci* 4:397
- Duan L, Dietrich D, Ng CH, Chan PMY, Bhalerao R, Bennett MJ, Dinneny JR (2013) Endodermal ABA signaling promotes lateral root quiescence during salt stress in Arabidopsis seedlings. *Plant Cell* 25:324–341
- Duan L, Sebastian J, Dinneny JR (2015) Salt-stress regulation of root system growth and architecture in Arabidopsis seedlings. In: *Plant cell expansion*. Springer, pp 105–122
- Feng S, Yue R, Tao S, Yang Y, Zhang L, Xu M, Wang H, Shen C (2015) Genome-wide identification, expression analysis of auxin-responsive GH3 family genes in maize (*Zea mays* L.) under abiotic stresses. *J Integr Plant Biol* 57:783–795
- Galvan-Ampudia CS, Testerink C (2011) Salt stress signals shape the plant root. *Curr Opin Plant Biol* 14:296–302
- Galvan-Ampudia CS, Julkowska MM, Darwish E, Gandullo J, Korver RA, Brunoud G, Haring MA, Munnik T, Vernoux T, Testerink C (2013) Halotropism is a response of plant roots to avoid a saline environment. *Curr Biol* 23:2044–2050
- Gälweiler L, Guan C, Müller A, Wisman E, Mendgen K, Yephremov A, Palme K (1998) Regulation of polar auxin transport by AtPIN1 in Arabidopsis vascular tissue. *Science* 282:2226–2230
- Gao Y, Zhao Y (2014) Auxin biosynthesis and catabolism. In: *Auxin and its role in plant development*. Springer, pp 21–38
- Geisler M, Murphy AS (2006) The ABC of auxin transport: the role of p-glycoproteins in plant development. *FEBS Lett* 580:1094–1102
- Geng Y, Wu R, Wee CW, Xie F, Wei X, Chan PMY, Tham C, Duan L, Dinneny JR (2013) A spatio-temporal understanding of growth regulation during the salt stress response in Arabidopsis. *Plant Cell* 25:2132–2154
- Guilfoyle TJ (2015) The PB1 domain in auxin response factor and Aux/IAA proteins: a versatile protein interaction module in the auxin response. *Plant Cell* 27:33–43
- Hagen G, Guilfoyle T (1985) Rapid induction of selective transcription by auxins. *Mol Cell Biol* 5:1197–1203
- Hsiao TC, Acevedo E, Fereres E, Henderson D (1976) Water stress, growth, and osmotic adjustment. *Philos Trans R Soc B Biol Sci* 273:479–500
- Iglesias MJ, Terrile MC, Bartoli CG, D'Ippólito S, Casalougué CA (2010) Auxin signaling participates in the adaptative response against oxidative stress and salinity by interacting with redox metabolism in Arabidopsis. *Plant Mol Biol* 74:215–222
- Iglesias MJ, Terrile MC, Windels D, Lombardo MC, Bartoli CG, Vazquez F, Estelle M, Casalougué CA (2014) MiR393 regulation of auxin signaling and redox-related components during acclimation to salinity in Arabidopsis. *PLoS ONE* 9:e107678
- Im Kim J, Baek D, Park HC, Chun HJ, Oh D-H, Lee MK, Cha J-Y, Kim W-Y, Kim MC, Chung WS, Bohnert HJ, Leeb SY, Bressan RA, Leed S-W, Yun D-J (2013) Overexpression of Arabidopsis YUCCA6 in potato results in high-auxin developmental phenotypes and enhanced resistance to water deficit. *Mol Plant* 6:337–349
- Jacobs M, Rubery PH (1988) Naturally occurring auxin transport regulators. *Science* 241:346–349
- Jung H, Lee D-K, Do Choi Y, Kim J-K (2015) OsIAA6, a member of the rice Aux/IAA gene family, is involved in drought tolerance and tiller outgrowth. *Plant Sci* 236:304–312
- Ke Q, Wang Z, Ji CY, Jeong JC, Lee H-S, Li H, Xu B, Deng X, Kwak S-S (2015) Transgenic poplar expressing Arabidopsis YUCCA6 exhibits auxin-overproduction phenotypes and increased tolerance to abiotic stress. *Plant Physiol Biochem* 94:19–27
- Kinoshita N, Wang H, Kasahara H, Liu J, MacPherson C, Machida Y, Kamiya Y, Hannah MA, Chua N-H (2012) IAA-Ala Resistant3, an evolutionarily conserved target of miR167, mediates Arabidopsis root architecture changes during high osmotic stress. *Plant Cell* 24:3590–3602
- Korasick DA, Westfall CS, Lee SG, Nanao MH, Dumas R, Hagen G, Guilfoyle TJ, Jez JM, Strader LC (2014) Molecular basis for AUXIN RESPONSE FACTOR protein interaction and the control of auxin response repression. *Proc Natl Acad Sci* 111:5427–5432
- Korasick DA, Jez JM, Strader LC (2015) Refining the nuclear auxin response pathway through structural biology. *Curr Opin Plant Biol* 27:22–28
- Kramer EM, Bennett MJ (2006) Auxin transport: a field in flux. *Trends Plant Sci* 11:382–386
- Le J, Liu XG, Yang KZ, Chen XL, Zou JJ, Wang HZ, Wang M, Vanneste S, Morita M, Tasaka M, Ding ZJ, Friml J, Beeckman T, Sack F (2014) Auxin transport and activity regulate stomatal patterning and development. *Nat Commun*. doi:10.1038/ncomms4090
- Lee M, Jung J-H, Han D-Y, Seo PJ, Park WJ, Park C-M (2012) Activation of a flavin monooxygenase gene YUCCA7 enhances drought resistance in Arabidopsis. *Planta* 235:923–938
- Liu L, Guo G, Wang Z, Ji H, Mu F, Li X (2014) Auxin in plant growth and stress responses. In: *Phytohormones: a window to metabolism, signaling and biotechnological applications*. Springer, pp 1–35
- Liu W, Li R-J, Han T-T, Cai W, Fu Z-W, Lu Y-T (2015) Salt stress reduces root meristem size by nitric oxide-mediated modulation of auxin accumulation and signaling in Arabidopsis. *Plant Physiol* 168:343–356
- Löfke C, Dünser K, Scheuring D, Kleine-Vehn J (2015) Auxin regulates SNARE-dependent vacuolar morphology restricting cell size. *Elife* 4:e05868

- Ludwig-Müller J (2011) Auxin conjugates: their role for plant development and in the evolution of land plants. *J Exp Bot* 62:1757–1773
- Mashiguchi K, Tanaka K, Sakai T, Sugawara S, Kawaide H, Natsume M, Hanada A, Yaeno T, Shirasu K, Yao H, McSteed P, Zhaoe Y, Hayashif K-I, Kamiyaa Y, Kasahara H (2011) The main auxin biosynthesis pathway in Arabidopsis. *Proc Natl Acad Sci* 108:18512–18517
- Mittler R, Blumwald E (2010) Genetic engineering for modern agriculture: challenges and perspectives. *Annu Rev Plant Biol* 61:443–462
- Morgan PW, Drew MC (1997) Ethylene and plant responses to stress. *Physiol Plant* 100:620–630
- Moubayidin L, Di Mambro R, Sabatini S (2009) Cytokinin–auxin crosstalk. *Trends Plant Sci* 14:557–562
- Nakayama N, Smith RS, Mandel T, Robinson S, Kimura S, Boudaoud A, Kuhlemeier C (2012) Mechanical regulation of auxin-mediated growth. *Curr Biol* 22:1468–1476
- Nanao MH, Vinos-Poyo T, Brunoud G, Thévenon E, Mazzoleni M, Mast D, Lainé S, Wang S, Hagen G, Li H, Guilfoyle TJ, Percy F, Vernoux T, Dumas R (2014) Structural basis for oligomerization of auxin transcriptional regulators. *Nat Commun*. doi:10.1038/ncomms4617
- Nir I, Moshelion M, Weiss D (2014) The Arabidopsis GIBBERELLIN METHYL TRANSFERASE 1 suppresses gibberellin activity, reduces whole-plant transpiration and promotes drought tolerance in transgenic tomato. *Plant, Cell Environ* 37:113–123
- Park J-E, Park J-Y, Kim Y-S, Staswick PE, Jeon J, Yun J, Kim S-Y, Kim J, Lee Y-H, Park C-M (2007) GH3-mediated auxin homeostasis links growth regulation with stress adaptation response in Arabidopsis. *J Biol Chem* 282:10036–10046
- Park J, Kim Y-S, Kim S-G, Jung J-H, Woo J-C, Park C-M (2011) Integration of auxin and salt signals by the NAC transcription factor NTM2 during seed germination in Arabidopsis. *Plant Physiol* 156:537–549
- Peer WA, Blakeslee JJ, Yang H, Murphy AS (2011) Seven things we think we know about auxin transport. *Mol Plant* 4:487–504
- Peer WA, Cheng Y, Murphy AS (2013) Evidence of oxidative attenuation of auxin signalling. *J Exp Bot* 64:2629–2639
- Peleg Z, Blumwald E (2011) Hormone balance and abiotic stress tolerance in crop plants. *Curr Opin Plant Biol* 14:290–295
- Peleg Z, Reguera M, Tumimbang E, Walia H, Blumwald E (2011) Cytokinin-mediated source/sink modifications improve drought tolerance and increase grain yield in rice under water-stress. *Plant Biotechnol J* 9:747–758
- Péret B, Li G, Zhao J, Band LR, Voß U, Postaire O, Luu D-T, Da Ines O, Casimiro I, Lucas M, Wells DM, Lazzerini L, Nacry P, King JR, Jensen OE, Schäffner AR, Maurel C, Bennett MJ (2012) Auxin regulates aquaporin function to facilitate lateral root emergence. *Nat Cell Biol* 14:991–998
- Petrásek J, Friml J (2009) Auxin transport routes in plant development. *Development* 136:2675–2688
- Ranocha P, Dima O, Nagy R, Felten J, Corratgé-Faillie C, Novák O, Morreel K, Lacombe B, Martinez Y, Pfrunder S, Jin X, Renou J-P, Thibaud J-B, Ljung K, Fischer U, Martinoia E, Boerjan W, Goffner D (2013) Arabidopsis WAT1 is a vacuolar auxin transport facilitator required for auxin homeostasis. *Nat Commun*. doi:10.1038/ncomms3625
- Rayle DL, Cleland RE (1992) The acid growth theory of auxin-induced cell elongation is alive and well. *Plant Physiol* 99:1271–1274
- Reinhardt D, Pesce E-R, Stieger P, Mandel T, Baltensperger K, Bennett M, Traas J, Friml J, Kuhlemeier C (2003) Regulation of phyllotaxis by polar auxin transport. *Nature* 426:255–260
- Robbins NE, Dinneny JR (2015) The divining root: moisture-driven responses of roots at the micro-and macro-scale. *J Exp Bot* 66:2145–2154
- Rosquete MR, Kleine-Vehn J (2013) Halotropism: turning down the salty date. *Curr Biol* 23:927–929
- Salehin M, Bagchi R, Estelle M (2015) SCFTIR1/AFB-based auxin perception: mechanism and role in plant growth and development. *Plant Cell* 27:9–19
- Schroeder JI, Kwak JM, Allen GJ (2001) Guard cell abscisic acid signalling and engineering drought hardiness in plants. *Nature* 410:327–330
- Shi H, Chen L, Ye T, Liu X, Ding K, Chan Z (2014) Modulation of auxin content in Arabidopsis confers improved drought stress resistance. *Plant Physiol Biochem* 82:209–217
- Shkolnik D, Krieger G, Nuriel R, Fromm H (2016) Hydrotropism: root bending does not require auxin redistribution. *Mol Plant*. doi:10.1016/j.molp.2016.02.001
- Shkolnik-Inbar D, Bar-Zvi D (2010) ABI4 mediates abscisic acid and cytokinin inhibition of lateral root formation by reducing polar auxin transport in Arabidopsis. *Plant Cell* 22:3560–3573
- Skirycz A, Inzé D (2010) More from less: plant growth under limited water. *Curr Opin Biotechnol* 21:197–203
- Song Y, Wang L, Xiong L (2009) Comprehensive expression profiling analysis of OsIAA gene family in developmental processes and in response to phytohormone and stress treatments. *Planta* 229:577–591
- Staswick PE, Serban B, Rowe M, Tiryaki I, Maldonado MT, Maldonado MC, Suza W (2005) Characterization of an Arabidopsis enzyme family that conjugates amino acids to indole-3-acetic acid. *Plant Cell* 17:616–627
- Stepanova AN, Yun J, Likhacheva AV, Alonso JM (2007) Multilevel interactions between ethylene and auxin in Arabidopsis roots. *Plant Cell* 19:2169–2185
- Stepanova AN, Robertson-Hoyt J, Yun J, Benavente LM, Xie D-Y, Doležal K, Schlereth A, Jürgens G, Alonso JM (2008) TAA1-mediated auxin biosynthesis is essential for hormone crosstalk and plant development. *Cell* 133:177–191
- Steudle E, Zimmermann U, Lüttge U (1977) Effect of turgor pressure and cell size on the wall elasticity of plant cells. *Plant Physiol* 59:285–289
- Sun F, Zhang W, Hu H, Li B, Wang Y, Zhao Y, Li K, Liu M, Li X (2008) Salt modulates gravity signaling pathway to regulate growth direction of primary roots in Arabidopsis. *Plant Physiol* 146:178–188
- Swarup R, Friml J, Marchant A, Ljung K, Sandberg G, Palme K, Bennett M (2001) Localization of the auxin permease AUX1 suggests two functionally distinct hormone transport pathways operate in the Arabidopsis root apex. *Genes Dev* 15:2648–2653
- Takeda S, Matsuoka M (2008) Genetic approaches to crop improvement: responding to environmental and population changes. *Nat Rev Genet* 9:444–457
- Tao Y, Ferrer J-L, Ljung K, Pojer F, Hong F, Long JA, Li L, Moreno JE, Bowman ME, Ivans LJ, Cheng Y, Lim J, Zhao Y, Ballaré CL, Sandberg G, Noel JP, Chory J (2008) Rapid synthesis of auxin via a new tryptophan-dependent pathway is required for shade avoidance in plants. *Cell* 133:164–176
- Tivendale ND, Ross JJ, Cohen JD (2014) The shifting paradigms of auxin biosynthesis. *Trends Plant Sci* 19:44–51
- Ulmasov T, Murfett J, Hagen G, Guilfoyle TJ (1997) Aux/IAA proteins repress expression of reporter genes containing natural and highly active synthetic auxin response elements. *Plant Cell* 9:1963–1971
- Vanderhoef LN, Dute RR (1981) Auxin-regulated wall loosening and sustained growth in elongation. *Plant Physiol* 67:146–149

- Wang R, Estelle M (2014) Diversity and specificity: auxin perception and signaling through the TIR1/AFB pathway. *Curr Opin Plant Biol* 21:51–58
- Wang Y, Li K, Li X (2009) Auxin redistribution modulates plastic development of root system architecture under salt stress in *Arabidopsis thaliana*. *J Plant Physiol* 166:1637–1645
- Wang S, Bai Y, Shen C, Wu Y, Zhang S, Jiang D, Guilfoyle TJ, Chen M, Qi Y (2010) Auxin-related gene families in abiotic stress response in *Sorghum bicolor*. *Funct Integr Genomics* 10:533–546
- Wang B, Chu J, Yu T, Xu Q, Sun X, Yuan J, Xiong G, Wang G, Wang Y, Li J (2015) Tryptophan-independent auxin biosynthesis contributes to early embryogenesis in *Arabidopsis*. *Proc Natl Acad Sci* 112:4821–4826
- Weiss D, Ori N (2007) Mechanisms of cross talk between gibberellin and other hormones. *Plant Physiol* 144:1240–1246
- Went FW, Thimann KV (1937) *Phytohormones*. MacMillan, New York
- West G, Inzé D, Beemster GT (2004) Cell cycle modulation in the response of the primary root of *Arabidopsis* to salt stress. *Plant Physiol* 135:1050–1058
- Wiśniewska J, Xu J, Seifertová D, Brewer PB, Růžička K, Blilou I, Rouquié D, Benková E, Scheres B, Friml J (2006) Polar PIN localization directs auxin flow in plants. *Science* 312:883
- Won C, Shen X, Mashiguchi K, Zheng Z, Dai X, Cheng Y, Kasahara H, Kamiya Y, Chory J, Zhao Y (2011) Conversion of tryptophan to indole-3-acetic acid by TRYPTOPHAN AMINOTRANSFERASES OF ARABIDOPSIS and YUCCAs in *Arabidopsis*. *Proc Natl Acad Sci* 108:18518–18523
- Woodward AW, Bartel B (2005) Auxin: regulation, action, and interaction. *Ann Bot* 95:707–735
- Yamada M, Greenham K, Prigge MJ, Jensen PJ, Estelle M (2009) The TRANSPORT INHIBITOR RESPONSE2 gene is required for auxin synthesis and diverse aspects of plant development. *Plant Physiol* 151:168–179
- Zažímalová E, Murphy AS, Yang H, Hoyerová K, Hošek P (2010) Auxin transporters: why so many? *Cold Spring Harb Perspect Biol* 2:a001552
- Zhang S-W, Li C-H, Cao J, Zhang Y-C, Zhang S-Q, Xia Y-F, Sun D-Y, Sun Y (2009) Altered architecture and enhanced drought tolerance in rice via the down-regulation of indole-3-acetic acid by TLD1/OsGH3.13 activation. *Plant Physiol* 151:1889–1901
- Zhang J-Y, He S-B, Li L, Yang H-Q (2014) Auxin inhibits stomatal development through MONOPTEROS repression of a mobile peptide gene STOMAGEN in mesophyll. *Proc Natl Acad Sci* 111:E3015–E3023
- Zhao Y (2012) Auxin biosynthesis: a simple two-step pathway converts tryptophan to indole-3-acetic acid in plants. *Mol Plant* 5:334–338
- Zhao Y, Christensen SK, Fankhauser C, Cashman JR, Cohen JD, Weigel D, Chory J (2001) A role for flavin monooxygenase-like enzymes in auxin biosynthesis. *Science* 291:306–309
- Zhao Y, Wang T, Zhang W, Li X (2011) SOS3 mediates lateral root development under low salt stress through regulation of auxin redistribution and maxima in *Arabidopsis*. *New Phytol* 189:1122–1134
- Zhu J-K (2002) Salt and drought stress signal transduction in plants. *Annu Rev Plant Biol* 53:247
- Zolla G, Heimer YM, Barak S (2009) Mild salinity stimulates a stress-induced morphogenic response in *Arabidopsis thaliana* roots. *J Exp Bot* 61:211–224
- Zwack PJ, Rashotte AM (2015) Interactions between cytokinin signalling and abiotic stress responses. *J Exp Bot* 66:4863–4871
- Zwiewka M, Nodzyński T, Robert S, Vanneste S, Friml J (2015) Osmotic stress modulates the balance between exocytosis and clathrin-mediated endocytosis in *Arabidopsis thaliana*. *Mol Plant* 8:1175–1187