Mechanisms regulating auxin action during fruit development

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Abstract

Auxin controls many aspects of fruit development, including fruit set and growth, ripening and abscission. However, the mechanisms by which auxin regulates these processes are still poorly understood. While it is generally agreed that precise spatial and temporal control of auxin distribution and signaling are required for fruit development, the dynamics of auxin biosynthesis and the mechanisms for its transport to different fruit tissues are mostly unknown. Despite major advances in elucidating many aspects of auxin biology in vegetative tissues, until recently, the nature and importance of auxin metabolism, transport and signaling during fruit ontogeny remained obscure. In this review, we summarize recent research that has started to elucidate the molecular mechanisms by which auxin is produced and transported in the fruit and to unravel the complexity of auxin signaling during fruit development. We also discuss recent approaches used to reveal the genes and regulatory networks that mediate cell and tissue-specific control of auxin levels in the developing fruit.

Abbreviations

- 4-Cl-IAA
  - 4-chloroindole 3-acetic acid
- ARF
  - auxin response factors
- DGT
  - diageotropica
- GA
  - gibberellin
- IAA
  - indole-3-acetic acid
- LCM
  - laser capture microdissection
- NPA
  - *N*-1-napthylphthalamic acid
- RNAi
  - RNA interference

Introduction

The successful initiation and development of fruit is a critical component of plant fitness. Accordingly, there is tremendous diversity in fruit form amongst angiosperms reflecting the evolutionary pressures that influence reproductive strategy (Knapp and Litt 2013). For example, Arabidopsis and other Brassicaceae produce dry dehiscent siliques in contrast to tomato (*Solanum lycopersicum*), which produces fleshy berry-type fruit. In general, fruit formation occurs after successful pollination and fertilization of the ovary, which triggers the rapid enlargement of the surrounding carpel tissue, first via cell division and then later via cell enlargement before the fruit undergoes ripening to facilitate seed dispersal (Seymour et al. 2013).

Auxin plays a critical role in fruit development, beginning with flower formation and patterning of the gynoecium, through fruit set, fruit growth and ripening (de Jong et al. 2009b, Sundberg and Østergaard 2009, Ruan et al. 2012). However, many details concerning the biosynthesis, distribution and activity of auxin in fruit remain unknown. In this review we summarize the current knowledge of auxin action during the different stages of fruit development with an emphasis in the molecular mechanisms governing auxin homeostasis. Although Arabidopsis research has begun to uncover the mechanisms underlying auxin control of fruit development (Sundberg and Østergaard 2009), we will highlight information acquired through research in tomato, an economically important fruit crop, and model system for studies

of fleshy fruits (Giovannoni 2004, Kimura and Sinha 2008). We also discuss recent approaches to help unravel the complexity of mechanisms that mediate cell and tissue-specific control of auxin levels and action in the developing fruit.

Role of auxin in fruit set and growth and sources of auxin

Various lines of evidence suggest that auxin regulates the transition of the ovary into a rapidly growing fruit. First, exogenous application of auxin to ovaries is able to bypass the requirement of pollination and fertilization for fruit set leading to the formation of parthenocarpic (seedless) fruit (Gustafson 1936, Vivian-Smith and Koltunow 1999, Serrani et al. 2008). This phenotype can be mimicked by expression in the ovary of genes encoding bacterial auxin biosynthetic enzymes, genes that confer high auxin sensitivity (Rotino et al. 1997, Acciarri et al. 2002, Carmi et al. 2003) or by disrupting auxin transport in un-pollinated ovaries (Kim et al. 1992, Dorcey et al. 2009, Serrani et al. 2010). Parthenocarpy may also be triggered by altering auxin signal transduction through downregulation or mutation of auxin response regulators, such as auxin response factors (ARFs) or Aux/IAA proteins (Wang et al. 2005, Goetz et al. 2007, de Jong et al. 2009a). Additionally, a fertilization-triggered increase in auxin response has been described in Arabidopsis (Dorcey et al. 2009, Fuentes and Vivian-Smith 2009). Such genetic and physiological data has led to a model of fruit initiation whereby a transient increase in auxin levels in the ovary after fertilization activates auxin signaling and modulates the expression of downstream target genes to promote fruit growth (de Jong et al. 2009b). However, quantitative data supporting a burst in auxin upon fertilization are scarce (Uchiumi and Okamoto 2010, Mariotti et al. 2011) and the molecular basis and tissue specificity of the increase in auxin content are still unknown.

Auxin is also believed to promote continued fruit expansion (Gillaspy et al. 1993) though much less is known about auxin regulation of cell division and enlargement following fruit set. In the majority of angiosperm species, the formation of seeds is intimately linked with fruit growth and development. Concentrations of indole-3-acetic acid (IAA), the main endogenous auxin, are higher in the seeds than the other fruit tissues in a diverse range of species (Varga and Bruinsma 1976, Mapelli et al. 1978, Devoghalaere et al. 2012, Pattison and Catalá 2012). Although high auxin levels in seed tissues are associated with the development of the embryo and endosperm, it has been suggested that the seeds are a source of auxin that diffuses, or is transported to, other fruit tissues where it promotes growth by cell division and expansion (Nitsch 1950, Ozga et al. 2002, Tiwari et al. 2013). Final fruit size is largely determined by seed number, and in some cases it has been shown that a peak in auxin concentration coincides with an increased rate of cell elongation, and that both are absent in seedless fruit (Tiwari et al. 2013).

A role for auxin as a regulator of fruit maturation has also been suggested (Trainotti et al. 2007, McAtee et al. 2013). During silique maturation in Arabidopsis, auxin transported away from the valve margins produces a local auxin minimum at the separation layer that is required for dehiscence (Sorefan et al. 2009). A decline in auxin levels has been associated with the onset of ripening in fleshy fruits (Böttcher et al. 2010, Symons et al. 2012) and may be required for ripening-related processes to occur (Aharoni et al. 2002, Castillejo et al. 2004, Ireland et al. 2013, Schaffer et al. 2013).

Auxin activity during the different stages of fruit development is regulated via its biosynthesis, metabolism and transport. However, until recently there has been a remarkable lack of information about the auxin biosynthetic pathway(s) in the fruit and the molecular basis for establishing local auxin maxima and gradients in fruit tissues.

Auxin biosynthesis and metabolism

As outlined above, the seeds are a probable site of de novo auxin biosynthesis during fruit development. However, data on the pathways and genes involved remain scarce. Recent studies using Arabidopsis suggest that the predominant route of auxin biosynthesis consists of a two-step linear pathway in which tryptophan is converted to indole 3-pyruvic acid by TAA/TAR enzymes and then to IAA by YUCCA enzymes (Stepanova et al. 2011, Won et al. 2011, Dai et al. 2013). A number of studies in different species show that *TAA*/*TAR* and *YUCCA* gene expression is high in seed tissues and correlates with auxin accumulation (Gallavotti et al. 2008, LeClere et al. 2010, Abu-Zaitoon et al. 2012, Bernardi et al. 2012), suggesting that this pathway is also likely dominant in fruit. In Arabidopsis embryos, *TAA1* and several

YUCCA genes show overlapping expression profiles during embryo development and their mutagenesis leads to developmental defects (Cheng et al. 2007, Stepanova et al. 2008). Along with its role in embryogenesis, de novo auxin biosynthesis is important for endosperm development, as demonstrated by the *de18* mutant of maize, which is caused by defects in ZmYUC1, and shows reductions in endosperm ploidy level, cell number, cell size and dry mass (Bernardi et al. 2012).

Evidence for auxin biosynthesis in the fruit tissues that surround the seeds is less clear. A tomato *TAA*/*TAR* gene, *SlTAR2*, is expressed preferentially during the cell expansion stage of fruit development in the seed and locular jelly, the fruit tissues with the highest auxin content (Pattison et al., unpublished data). In addition to *SlTAR2*, members of the tomato *YUCCA* family (known in tomato as *toFZY*), particularly *toFZY6*, show preferential expression in seed tissues (Fig. 1; Expósito-Rodríguez et al. 2011). Some *YUCCA* genes are also expressed in external pericarp tissue (Expósito-Rodríguez et al. 2011, Mariotti et al. 2011) and *toFZY4* shows maximal expression in ripe fruit with higher expression in pericarp relative to seed tissue (Fig. 1; Expósito-Rodríguez et al. 2011).

Alternative pathways of auxin biosynthesis may also be active in fruit depending on the species and developmental stage. For example, *TAA*/*TAR* and *YUCCA* genes show very low levels of expression in mature apple fruit, but there is strong expression of the *AMI* genes, *AMI1* and *AMI101*, which encode indole-3-acetamide hydrolase, an enzyme participating in an alternative tryptophan-dependent auxin biosynthesis pathway which uses indole-3-acetamide as an intermediate (Schaffer et al. 2013).

Furthermore, endogenous auxins other than IAA may participate in fruit development in some species. For example, in pea seeds 4-chloroindole 3-acetic acid (4-Cl-IAA) accumulates to much higher levels than IAA throughout the whole period of fruit development except for the first few days after pollination (Tivendale et al. 2012). 4-Cl-IAA is produced from 4-chlorotryptophan via 4-chloroindole 3-pyruvic acid in a pathway parallel to the TAA/TAR–YUCCA pathway of IAA biosynthesis (Tivendale et al. 2012) and is found widely in the Vicieae and Fabaceae families (Reinecke 1999).

Conjugation of IAA to amino acids, catalyzed by the auxin-inducible GH3 amido synthetases, may act as an additional mechanism in the control of auxin homeostasis (Staswick et al. 2005) and the complex role of these proteins in diverse plant developmental processes is just emerging. The expression of *GH3* genes has been associated with the ripening of some fruit such as pepper (Liu et al. 2005), grape berries (Böttcher et al. 2010), tomato (Kumar et al. 2012) and apple (Devoghalaere et al. 2012) and the conjugation of IAA with aspartic acid has been suggested as a mechanism to keep a low concentration of free IAA during ripening (Böttcher et al. 2010, Böttcher et al. 2011).

It has been suggested that auxin biosynthesis may be controlled by cytokinin, which accumulates to high levels in ovaries (Matsuo et al. 2012). Exogenous application of either hormone is able to induce the biosynthesis of the other (Jones et al. 2010), and cytokinin-induced parthenocarpy in tomato increases both auxin concentration and *toFZY* expression (Ding et al. 2013).

Molecular components of polar auxin transport in the fruit

Polar auxin transport is a key regulator of auxin distribution across plant tissues and contributes to the formation of local auxin maxima and minima that control various aspects of plant growth and development (Petrášek and Friml 2009). The direction of auxin transport across organs and tissues is in large part controlled by the AUX/LAX and PIN-FORMED (PIN) family proteins that facilitate cellular auxin influx and efflux, respectively, and contribute to the formation of auxin gradients (Petrášek and Friml 2009).

In Arabidopsis, the roles of PIN and AUX/LAX transporters in vegetative development have been studied extensively but their function in fruit development is less clear. However some studies have revealed a function for specific *PIN* genes in distinct stages of Arabidopsis reproductive development: *PIN1* has been shown to play a role in female gametophytic development (Ceccato et al. 2013) and *PIN3* is expressed during fruit development and its localization is regulated at the valve margin to create a local auxin minimum (Sorefan et al. 2009). Recent studies in tomato, which contains 10 *PIN* and 5 *AUX*/*LAX* genes, have started to investigate their action during fruit development (Nishio et al. 2010, Mounet et al. 2012, Pattison and Catalá 2012). The majority of *PIN* and *AUX*/*LAX* genes in tomato are expressed

primarily in immature fruit and predominantly in the internal tissues between seed and pericarp (Nishio et al. 2010, Mounet et al. 2012, Pattison and Catalá 2012). *PIN1*, *PIN4*, *PIN8* and *LAX2* expression is found primarily in the placenta for a prolonged period from fruit set to the onset of ripening, whereas *PIN3* and *PIN9* are primarily expressed in the internal tissues of young fruit 5 days after anthesis (Pattison and Catalá 2012; Fig. 1). The importance of the placental tissue for auxin transport in tomato is demonstrated by the effect of *N*-1-naphthylphthalamic acid (NPA), an inhibitor of polar auxin transport, which causes increased auxin activity in the same internal placental tissues where *PIN* expression is highest (Pattison and Catalá 2012). The expression of these genes in immature tomato fruit coincides with a high rate of basipetal auxin transport to the parent plant via the pedicel which likely acts to prevent premature abscission during fruit development. The rate of auxin transport declines during ripening coinciding with decreased transporter-gene expression (Else et al. 2004, Pattison and Catalá 2012).

RNA interference (RNAi)-mediated silencing of the expression of tomato *PIN4*, the most highly expressed *PIN* gene in the placental tissue, is able to induce parthenocarpy, likely due to the accumulation of excess auxin in the ovary (Mounet et al. 2012), in a similar manner as has been reported when basipetal auxin transport has been reduced either by NPA treatment or by decapitation of the shoot apical meristem (Serrani et al. 2010). Although silencing of the same gene did not result in parthenocarpy in a previous study (Pattison and Catalá 2012), this may reflect differences between varieties or could be due to a compensatory effect caused by co-silencing of additional *PIN* genes.

A subset of auxin transporter genes exhibit different expression profiles during tomato fruit development. *LAX3* and *PIN7* both showed peak expression at the beginning of fruit ripening, and were primarily expressed in the pericarp (Pattison and Catalá 2012; Fig. 1). Two further exceptions include *PIN5* and *PIN6*, which showed highest expression in the seed/locular tissue and pericarp, respectively (Pattison and Catalá 2012; Fig. 1). Interestingly PIN5 and PIN6 are members of a divergent family subclade of PINs that in Arabidopsis are localized in the endoplasmic reticulum rather than the plasma membrane, and may therefore act in subcellular auxin compartmentalization which is another mechanism in which auxin concentration could be modulated (Barbez and Kleine-Vehn 2013).

Polar localization of PIN proteins at the plasma membrane controls the directionality of auxin fluxes and it is partially regulated by the phosphorylation status of the PIN proteins which depends on the action of the PID kinase and protein phosphatase PP2A (Petrášek and Friml 2009). In Arabidopsis, the basic helix-loop-helix transcription factor INDEHISCENT (IND) prevents PIN3 polar localization in the separation layer at the fruit valve margin, likely through regulation of *PID* expression (Sorefan et al. 2009).

In addition to PIN and AUX/LAX proteins, a subgroup of the ATP-Binding-Cassette (ABC) transporter family including Arabidopsis ABCB1, ABCB4 and ABCB19 also transport auxin, and both *ABCB1* and *ABCB19* show relatively strong expression in the developing silique (Titapiwatanakun and Murphy 2009). Interestingly, Arabidopsis *ABCB* expression may be influenced by the AUCSIA family of small proteins (Molesini et al. 2012), which in tomato have been implicated in the control of fruit set. Tomato *AUCSIA1* and *AUCSIA2* show decreased expression during fruit initiation and RNAi-mediated silencing of *AUCSIA* genes results in parthenocarpy and hyperaccumulation of auxin in the ovary (Molesini et al. 2009). Although it could not be determined whether this was due to differences in biosynthesis, transport or degradation, notably auxin transport was altered in the root.

Analysis of the expression patterns of genes involved in auxin transport, metabolism and biosynthesis as the described above for tomato are an important first step to elucidate the control of auxin levels and gradients in the fruit, and are being performed in an increasing number of diverse fruit species (Dal Cin et al. 2009, Kang et al. 2013). However there is still a lack of studies in which gene function is revealed through genetic evidence, particularly in fleshy fruit species. Similarly, more research is needed to elucidate the mechanisms regulating gene expression and/or the post-transcriptional regulation of auxin transport proteins in fruit development.

Auxin signaling in fruit development

The interaction of auxin with TIR1/AFB family F-box proteins promotes ubiquitin-ligase catalyzed degradation of Aux/IAA proteins, which in turn releases ARF transcription factors to regulate the transcription of target genes (Chapman and Estelle 2009). Recent analyses of the tomato genome suggest there are at least 3 *TIR1*/*AFB* (Ben-Gera et al. 2012), 25 *Aux*/*IAA* (Audran-Delalande et al. 2012) and 21

ARF genes (Wu et al. 2011), providing multiple possible combinations of isoforms that could modulate auxin response according to tissue type or developmental stage (Kieffer et al. 2010). There is genetic evidence suggesting that IAA9 and ARF7 in tomato and ARF8 in Arabidopsis are involved in the regulation of fruit set and that they act to restrict the expression of downstream targets of auxin prior to pollination (Wang et al. 2005, Goetz et al. 2007, de Jong et al. 2009a).

Recent data are also uncovering new roles for other Aux/IAA and ARF proteins in fruit growth after fruit set. It was recently demonstrated that tomato *IAA27* and *ARF10* are involved in the regulation of seed development and the size and shape of the fruit (Bassa et al. 2012, Hendelman et al. 2012). Silencing of *ARF4*, which is expressed strongly in tomato pericarp resulted in enhanced accumulation of starch and chlorophyll content, which suggests the involvement of auxin signaling in the control of chloroplastic activity and sugar metabolism in the fruit (Jones et al. 2002, Sagar et al. 2013).

The use of the auxin-inducible *DR5* promoter coupled to a reporter gene has allowed detailed spatial analysis of the auxin response during the different stages of fruit development, not only in Arabidopsis (Sorefan et al. 2009) but also in tomato (Pattison and Catalá 2012). Of interest in the future will be integrating the above mentioned phenotypes, and Aux/IAA and ARF activity, with the spatial localization of auxin induced gene expression as portrayed by *DR5* based reporter lines. Analysis of tomato fruit expressing the *DR5::RFP* reporter indicated that auxin induced gene expression was predominantly concentrated in the seeds and funiculus, the same tissues that had highest expression of auxin biosynthetic genes (Pattison and Catalá 2012). Surprisingly little *DR5* activity was evident in the pericarp even though auxin concentration was almost as high as in the internal tissues at 5 days after anthesis (Pattison and Catalá 2012). This suggests that auxin signaling in the pericarp may be poorly portrayed by *DR5* based reporters. The newly developed DII-VENUS auxin reporter system (Brunoud et al. 2012), which directly reports Aux/IAA degradation rather than the downstream activation of gene expression, is likely to offer further insights into the spatial organization of auxin signaling in the fruit.

Another component of auxin signaling in fruit is the cyclophilin diageotropica (DGT). The tomato *dgt* mutant shows a number of defects during early fruit development including reductions in seed number, the rate of fruit set and fruit size and increased time between anthesis and ripening (Balbi and Lomax 2003). The molecular mechanism by which DGT acts is unknown but it may function as a positive regulator of auxin signaling as mutation of this gene or chemical disruption of cyclophilin activity results in reduced expression of early auxin responsive genes (Oh et al. 2006, Lavy et al. 2012).

An important aspect of auxin regulation of early fruit development is cross talk with gibberellin (GA) signaling. Both pollination and exogenous auxin can induce the expression of tomato GA biosynthetic genes and auxin application to pea pericarp after the removal of seeds is sufficient to induce GA biosynthesis, supporting the hypothesis that auxin is transported from the seeds to the external fruit tissues (Serrani et al. 2008, Ozga et al. 2009). Interestingly, analysis of Arabidopsis mutants lacking DELLA proteins, which are key mediators of GA signaling, have shown that auxin-induced parthenocarpy is the result of activated GA signaling (Fuentes et al. 2012). Furthermore, ARF7, which is highly expressed in un-pollinated ovaries, is a key mediator of auxin-GA cross talk in tomato as its suppression resulted in much lower levels of several active GAs in the pericarp (de Jong et al. 2011). There is also clear evidence for synergism and interdependence between the auxin and brassinosteroid transcriptional responses in vegetative tissues (Hardtke et al. 2007) and, for example, in Arabidopsis seedlings integration of both pathways occurs through modulation of the activity of ARF2 by brassinosteroids (Vert et al. 2008). However, the relationship between the signaling pathways of these two phytohormones during fruit development has not yet been explored. Interestingly, brassinosteroid biosynthesis is necessary for fruit set in cucumber and exogenous application induces parthenocarpy probably by promoting cell division, reminiscent of auxin application (Fu et al. 2008). However, during ripening the two hormones may have opposite effects. In grape, endogenous biosynthesis and exogenous application of brassinosteroid promotes ripening (Symons et al. 2006) whereas it is delayed by auxin (Böttcher et al. 2010).

While auxin acts as a positive regulator during fruit set and fruit growth, it appears to act as a negative regulator during fruit maturation and ripening. It has been proposed that auxin produced by the seed in growing fruit acts to prevent ripening and premature dispersal before the seeds are fully developed (Sundberg and Østergaard 2009). Accordingly, a number of genes that are usually upregulated during ripening are repressed by auxin (Aharoni et al. 2002), including the strawberry SHATTERPROOF gene,

which encodes a MADS box transcription factor that controls a variety of ripening related events (Daminato et al. 2013). Although these studies emphasize the function of auxin as a repressor of genes involved in the ripening process, a more complex role of auxin regulating this process is currently emerging (Trainotti et al. 2007). The expression of certain auxin-related genes, including those encoding transporters, enzymes in the biosynthetic pathway and components of the auxin response pathway (Fig. 1) increases during the transition to ripening in tomato and peach (Trainotti et al. 2006, Expósito-Rodríguez et al. 2011, Pattison and Catalá 2012). While the functional significance of this remains unclear, it may point to tissue-specific or species-specific differences in auxin signaling during ripening. Indeed in peach, declining levels of auxin in the stone coincide with increasing levels of auxin and ethylene in the mesocarp (Trainotti et al. 2007, Tatsuki et al. 2013). The crosstalk between auxin and ethylene, the key regulator of ripening in climacteric fruit such as tomatoes, has been suggested by studies that show an ethylene-dependent accumulation of the tomato Aux/IAA gene *IAA3* during ripening (Chaabouni et al. 2009). Additionally, recent transcriptomic studies (Tomato Genome Consortium 2012) have shown that tomato *ARF2* expression increases during ripening. Interestingly, ethylene modulates the potential interaction of IAA3 with ARF2 (Salma et al. 2009), which suggests that ethylene could be modulating auxin responses during fruit ripening (Fig. 1).

Tissue-specific transcript profiling as a new approach to unravel the complexity of auxin action during fruit development

The diversity in fruit form and development across the angiosperms and the composite nature of fruit, consisting of multiple tissue types, presents unique challenges when attempting to understand the role of auxin. Hormonal regulation of fruit development in a model species such as Arabidopsis, which produces dry siliques, may not be equivalent to hormonal regulation of fleshy fruit development in tomato, and likewise auxin is likely to act differently in the diverse tissue types that make up the fruit.

Genome-scale expression profiling using next-generation sequencing (RNA-seq) combined with techniques to isolate individual fruit cell and tissue types such as laser capture microdissection (LCM; Schmid et al. 2012), offers the ability to study gene expression in fruit development with enhanced levels of spatial and temporal details. Moreover, these approaches are not restricted to traditional model organisms and can be applied to study natural diversity in fruit development. LCM has recently been used to obtain expression profiles of individual cell types in Citrus and tomato fruit (Matas et al. 2010, 2011). The use of this approach to study fruit development will not only allow a global analysis of the auxin pathway but will also reveal or highlight the tissue-specificity of its components: transport, biosynthesis and signaling. In addition, tissue-specific transcription maps along the different stages of fruit development can be used as a powerful tool to generate developmental regulatory networks as has been shown for other plant organs (Taylor-Teeples et al. 2011, Belmonte et al. 2013).

The potential of tissue-specific transcriptome profiling to improve understanding of auxin in fruit development was recently demonstrated in a study of gene expression in different tissues of woodland strawberry (Kang et al. 2013). Although this study relied on hand dissection, rather than LCM, the separation of tissues allowed conclusions to be made about the spatial localization of auxin-related gene expression. For example, a distinct group of *YUCCA* and *TAA/TAR* genes were identified that were predominantly expressed in the endosperm and seed coat while others were expressed in the embryo. The induction of these genes during fruit development suggests that the endosperm and seed coat are the main sites of auxin synthesis after fertilization (Kang et al. 2013).

As an example of how LCM coupled with RNA-seq can be used to explore auxin biology in fruit, we profiled the expression of the tomato ortholog of *BEL1* and a member of the *PLETHORA* (*PLT*) family, in a tissue-specific data set from tomato ovaries and fruit (Fig. 2). *BEL1* encodes a homeodomain transcription factor which in Arabidopsis is expressed in the primordia of developing ovules and integuments where it is required for correct patterning, and plays a key role in regulating the expression of *PIN1* (Reiser et al. 1995, Bencivenga et al. 2012). *PLETHORA* genes encode AP2 domain transcription factors that have been implicated in regulating the expression of *YUC1* and *YUC4* (Pinon et al. 2013). LCM was performed on cryosections of tomato ovaries at anthesis and fruit at 4 days post anthesis to collect the various different tissues (Fig. 2A). RNA-seq analysis of the microdissected tissues demonstrated that *BEL1* expression was largely restricted to ovules at anthesis and then the funiculus and seed coat at 4 days post anthesis, whereas the *PLT* family gene displayed strong expression in the funiculus (Fig. 2B). Interestingly these expression patterns overlap with the tissues that displayed

strongest *DR5* activity (Fig. 3) suggesting that in tomato, like in Arabidopsis, BEL and PLT transcription factors regulate components of auxin transport and biosynthesis, respectively.

Non-biased tissue-specific transcriptomic approaches have the potential to dramatically increase the discovery of rare and cell-type specific transcripts and will help identify regulatory networks controlling auxin homeostasis. This information will ultimately help to build a model integrating hormone regulated cell division and expansion and to identify pathways potentially critical to fruit set and growth.

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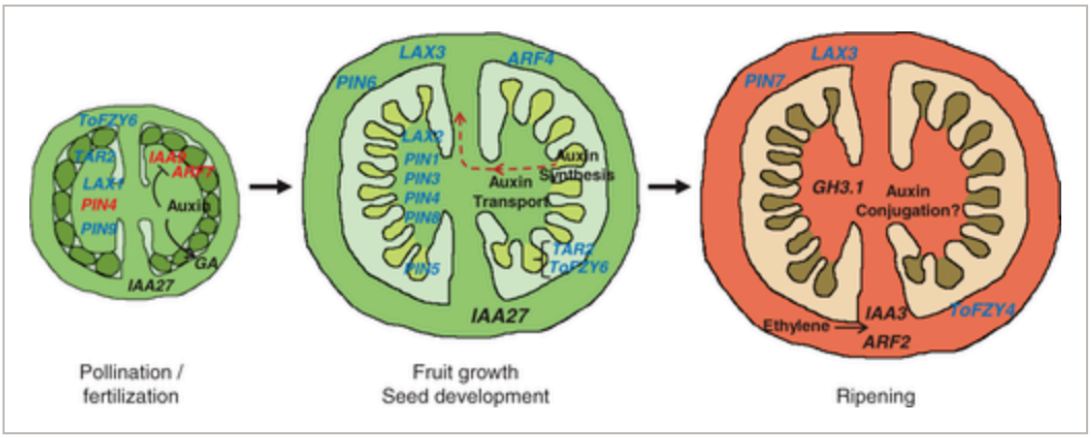

Figure 1. Mechanisms and genes regulating auxin action during tomato fruit development. Loss-of-function of genes in red cause parthenocarpy implying a role in the regulation of fruit set. Genes in blue display preferential expression in the fruit tissue they are placed. The tissue-specificity of genes in black is still unknown. In the unpollinated ovary, the auxin response is inhibited by ARF7 and IAA9 but upon fertilization, *ARF7* expression is downregulated and IAA9 is degraded. IAA27 may act to regulate the auxin response in ovaries and during fruit growth. *TAR2* and *toFZY6* which encode enzymes involved in auxin biosynthesis are strongly expressed in the ovary and the developing seed. Auxin is transported from the seed through the coordinated action of multiple PIN and AUX/LAX transporters in the placenta. PIN5 in the seeds, and PIN6 and LAX3 in the pericarp potentially mediate intracellular auxin compartmentalization or intercellular transport in these tissues. ARF4 is likely involved in mediating pericarp-specific auxin responses. During ripening, specific genes involved in auxin biosynthesis and transport, *toFZY4*, *LAX3* and *PIN7*, show increased expression in the pericarp. *GH3.1* which encodes an auxin conjugating enzyme also shows elevated expression at this stage. *ARF2* and *IAA3* expression increases possibly as a result of elevated ethylene levels.

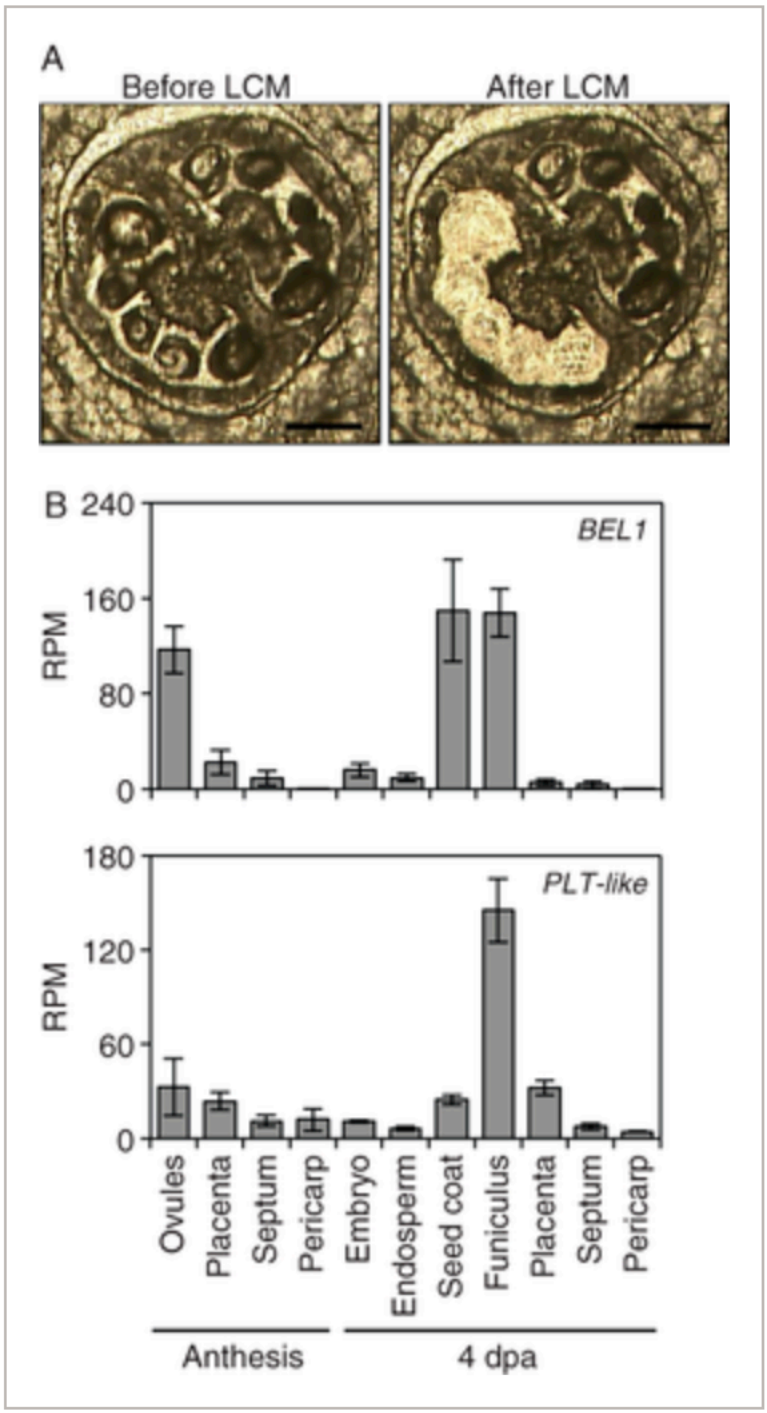

Figure 2. Laser capture microdissection for gene expression analysis in fruit. (A) Cryosection of a tomato ovary before and after laser capture microdissection. The image on the right was taken after removal of the ovules for RNA extraction. Scale bars = 200 μm. (B) Expression of *BEL1* and *PLETHORA* transcription factors in tomato ovary and fruit. Gene expression in microdissected tissues of the ovary and 4 days post anthesis fruit was analyzed by RNA-seq. Expression levels in each tissue are expressed as a normalized read count. RPM, reads per million reads mapped; dpa, days post anthesis.

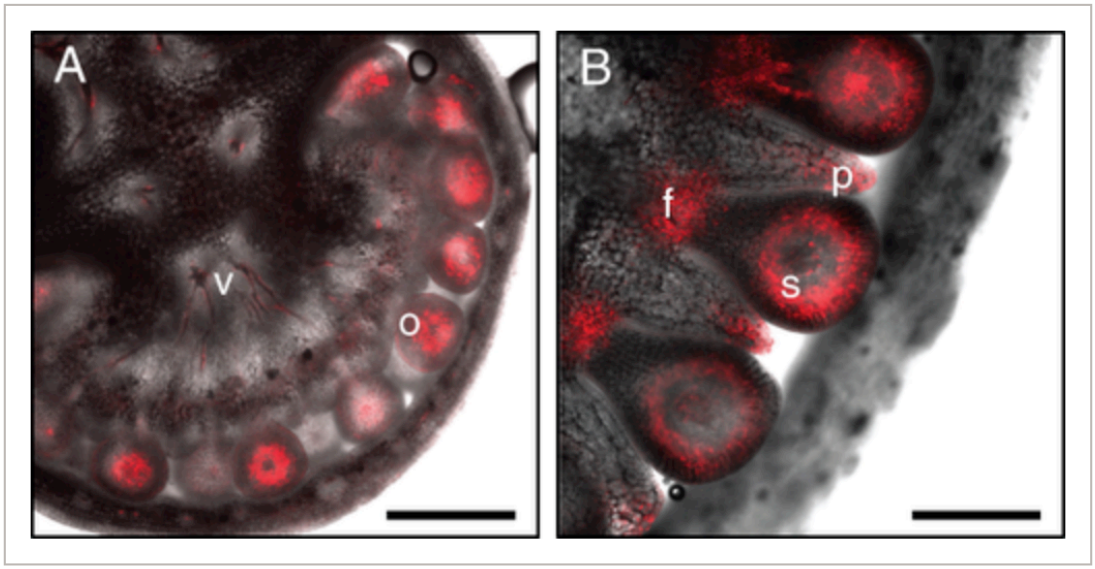

Figure 3. Auxin activity during early fruit development. Expression of the auxin responsive reporter *DR5rev::mRFPer* was imaged in ovaries at anthesis (A) and young fruit 6 days after anthesis (B). RFP fluorescence in ovules (o), vascular strands (v), seeds (s), funiculus (f) and placenta (p) is indicated. Scale bars = 400 µm.