6 Hormonal cues during fruit initiation

In 1936, Gustafson discovered that application of synthetic auxins to emasculated flowers of several different plant species resulted in parthenocarpic fruit development and, thus, established the initial linkage between fruit initiation and plant-growth regulators (Gustafson, 1936). At present, three main types of plant-growth regulators are recognized as having phytohormonal properties that can potentially induce fruit setting and fruit development (Gillaspy *et al.*, 1993). Application of auxin, gibberellins or cytokinin, either alone or in combination, has been shown to trigger parthenocarpy across a wide variety of plant species (Gustafson, 1936; King, 1947; Srinivasan and Morgan, 1996; Vivian-Smith andKoltunow, 1999; Ozga *et al.*, 2002, 2003). Application of optimal combinations of plant-growth regulators to emasculated pistils can often promote elongation to the extent observed in fully seeded fruits (Vivian-Smith *et al.*, 2001). These results have led to a long standing belief that fruit initiation is sustained by phytohormone biosynthesis occurring during the stages of seed development, although often this assertion remains unchallenged.

6.1 Auxin

- Auxin appears to have a primary role during fruit initiation since the genetic analysis of wild-type *Arabidopsis* fruit initiation with gibberellin biosynthesis and perception mutants shows that auxin-mediated differentiation underlies other signalling pathways.

- transcriptional profiling during fruit initiation also shows directionality in phytohormonal responses with auxin preceding gibberellin responses at 12–14 h period post-fertilization.

- The use of the transgenic *DEFH::iaaM* construct in a broad range of species additionally suggests a universal role for auxin in triggering fruit set.

Accordingly, auxin-mediated signalling is an early response in the Arabidopsis ovule (Fig. 4.2). Auxin-responsive reporters show transcriptional activation 2–3 h post-fertilization expression, when the nuclear endosperm has undergone only one division.

6.1.1 Auxin-mediated transcriptional activation

- Several genetic lesions in the auxin pathway conferring autonomous fruit initiation have been isolated. Each of these mutants appears to work within the auxin-mediated transcriptional network. The *Arabidopsis* genome encodes 22 functional auxin response transcription factors (ARFs) and 29 Aux/IAA interacting proteins, and each gene appears to have strong sequence conservation in other plant genomes.
- The ARFs are a family of Aux/IAA interacting proteins that contain a DNA-binding domain (DBD) which recognizes auxin response elements (AuxREs) in DNA sequences.
- *FWF/ARF8* transcripts are naturally downregulated within 24 h post-pollination.

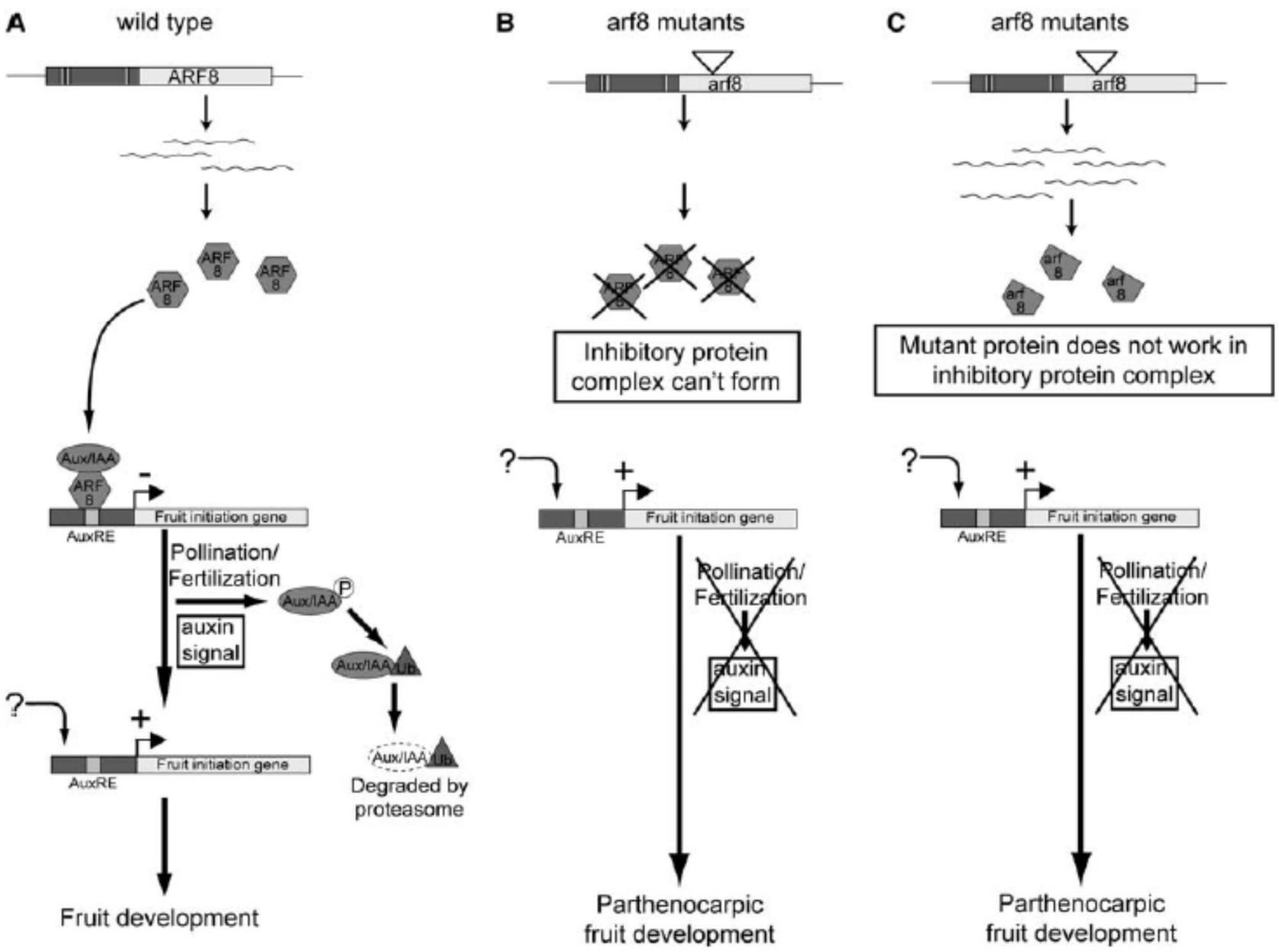

Figure 6. Model for the Role of *ARF8* in Fruit Development.

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AUXIN RESPONSE FACTOR8 Is a Negative Regulator of Fruit Initiation in *Arabidopsis* [W]

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ARF8:GUS expression is switched off soon after fertilization has occurred in wild-type plants, indicating that a fertilization signal deactivates ARF8. The removal of ARF8 activity after fertilization might abolish a developmental block that represses fruit growth and allows initiation of seed and fruit developmental programs.

In unfertilized ovules, expression of the ARF8:GUS marker persists and staining is observed throughout the ovule, indicating that the negative regulation through ARF8 is kept active. These expression patterns are therefore consistent with ARF8 acting as a negative regulator of fruit initiation, and collectively they indicate a central role for the ovules in mediating positive and negative signals involved in fruit development.

Model for the Role of ARF8 in Fruit Development

Figure 6 shows a model for the role of ARF8 during the transition from carpel to fruit growth. Our data suggest that ARF8 represses fruit development, and in the simplest model, ARF8 may do so by directly activating genes that themselves repress fruit development. Alternatively, ARF8 may invoke repression by being a member of a complex of proteins. Protoplast transformation experiments support the concept that the transcriptional activity of the ARF8 protein is regulated by heterodimerization with auxin/indole-3-acetic acid (Aux/IAA) proteins that inhibit this activity (Guilfoyle et al., 1998; Ulmasov et al., 1999a, 1999b; Guilfoyle and Hagen, 2001; Rogg and Bartel, 2001; Liscum and Reed, 2002; Tiwari et al., 2003). Physical interactions between ARF8 and members of both the Aux/IAA repressor and ARF protein families have been demonstrated (Hardtke et al., 2004;

6.1.2 Auxin biosynthesis and transport

- Three important processes that regulate auxin action in the flower and fruit are its biosynthesis, transport and catabolism.

iaaM is involved in the conversion of tryptophan to indole-3-acetamide, which is the hydrolyzed to IAA.

In an experiment, the *iaaM* gene from *Pseudomonas syringae* pv savastonoi was placed under the control of the placental and ovule-specific promoter DEFH9. Eggplants and tobacco transformed with this construct had parthenocarpic fruit development...

Experiments with PAT inhibitors show that flower morphology and apical–basal carpel polarity can be severely disrupted by PAT inhibitor application (Nemhauser *et al.*, 2000). Timed applications of PAT inhibitors can also induce parthenocarpy, suggesting that the anthesis ovules and pistil may exert control over PAT, either directly or indirectly, and this has an impact on the regulation of fruit initiation

This appears to contradict the proposed mechanism for auxin action in fruit initiation, since PAT is required to transport auxin from the ovule to the carpel to trigger growth and development. Therefore, the mechanism by which PAT inhibitors triggers fruit initiation may rely on a balance between PAT and AuxRE transcriptional activation.

- **To explain the apparent paradox**:

one must consider that auxin is also prevalent in the target cells. Therefore, the end concentration in target cells is important and may normally be restricted to a low level through potentiation of PAT and a reduction in transcriptional activation. Once PAT is inhibited, AuxRE would be activated due to the increased net auxin accumulation within the cell that would trigger fruit initiation through cross-talk with other phytohormonal pathways.

To explain PAT,

a chemiosmotic model was proposed in which the noncharged, lipophilic IAA molecule enters the cell through diffusion, or through the action of a saturable auxin import carrier.

Once inside the cell, the IAA molecule is deprotonated at the higher cytoplasmic pH and only can exit through active export by auxin efflux carriers (AECs) and endosomal/vessicle transport.

The specific location of AECs at the basal side of the cell was hypothesized to be the rate-limiting transport step of PAT.

The *PIN1* gene encodes a transmembrane protein that has similarity to bacterial-type transporters and data suggest that PIN proteins function as AECs.

The *PIN* family in *Arabidopsis* comprises eight members six of which have been functionally characterized through genetic analysis.

Genetic analysis has also uncovered partial redundancy and functional compensation amongst PIN family members. As a functional AEC, the PIN1 protein is localized to the basal end of xylem parenchyma and cambial cell files in the *Arabidopsis* inflorescence and root axis.

6.1.3 Auxin signalling and feedback regulation

- Auxin rapidly induces early auxin response genes including two other major classes, *SAURs* and *GH3s.*

The *GH3* gene family in *Arabidopsis* represents an important network component in feedback signalling, auxin homeostasis and in the homeostasis of jasmonic acid (Liu *et al.*, 2005; Terol *et al.*, 2006). GH3 enzymes catalyze bidirectional conjugations of indolic compounds and jasmonic acids with amino acids (Liu *et al.*, 2005). Many *GH3* genes are responsive to environmental stimuli, but most are also primary auxin response genes and are induced in response to auxin treatments to pistils and have altered expression in *fwf/arf8* and *arf6* mutant backgrounds (Nagpal *et al.*, 2005). The genes *GH3.5*, *GH3.6* and *GH3.17* appear to be targets of ARF8 and ARF6 (Tian *et al.*, 2004; Nagpal *et al.*, 2005) and plants overexpressing *ARF8* showed a decrease in free IAA content possibly due to *GH3* expression (Tian *et al.*, 2004). Regulation through *ARF8* and *GH3* genes at anthesis may therefore be a mechanism that constrains auxin responses further since free auxin would be removed and this would potentiate Aux/IAA proteins to form inactive complexes with ARF proteins.

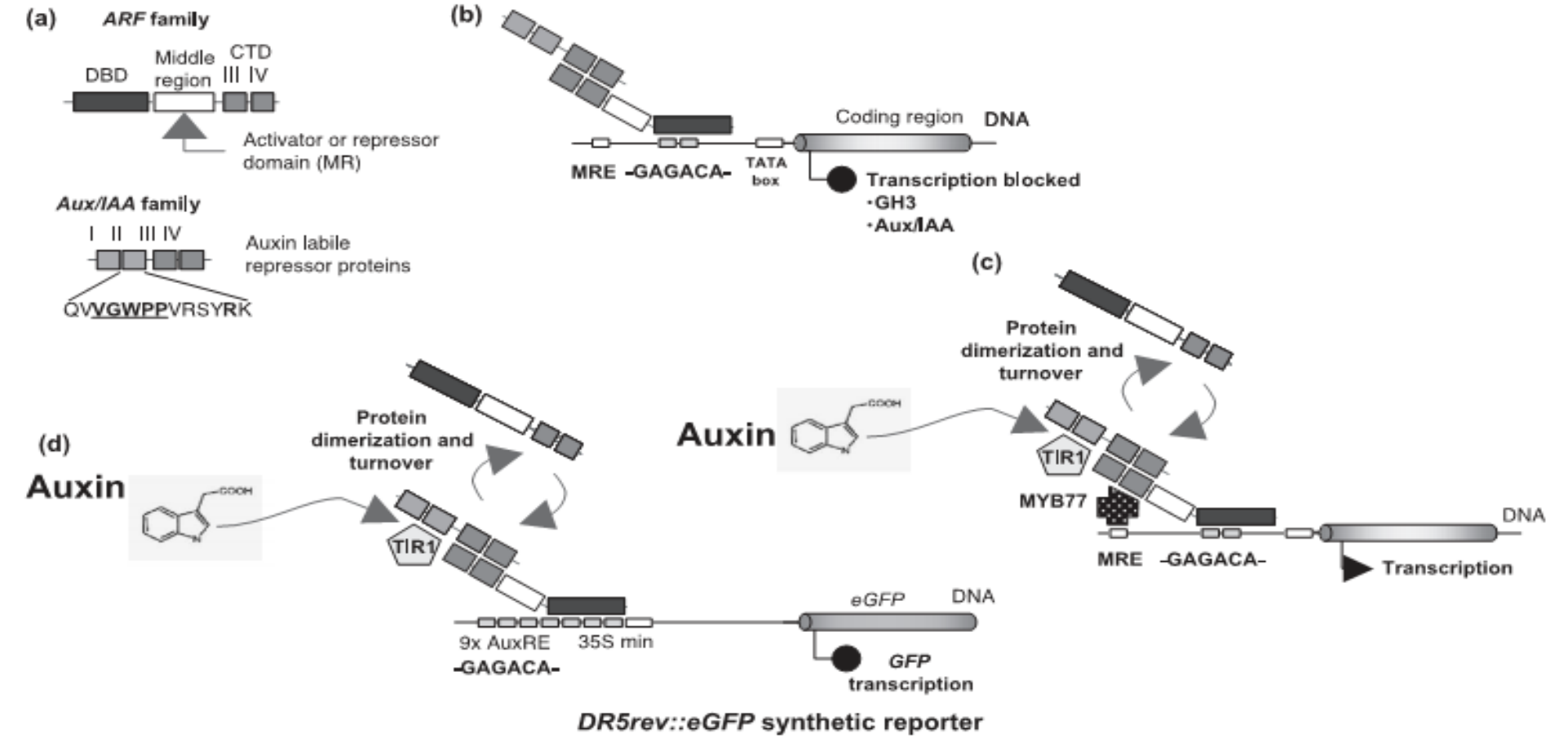


Figure 4.3 Auxin-responsive gene regulation. (a) The structure and function of ARF and Aux/IAA proteins which regulate expression of auxin-responsive genes. ARF proteins contain a DNA-binding domain (DBD) and carboxy-terminal domains (CTD) III and IV. The CTD regions facilitate hetero- and homodimerization amongst other ARF and Aux/IAA proteins as well as binding with MYB77. (b) Repression of downstream coding regions occurs when an Aux/IAA protein interacts with an ARF protein that is bound to auxin response elements (AuxREs). Downstream target genes are often Aux/IAA proteins and *GH3* genes creating a loop of auxin transcriptional responsiveness. (c) Possible transcriptional activation by ARF of auxin response genes containing an AuxRE after free auxin induces lability of the Aux/IAA protein through the TIR1/AFB proteolysis pathway. Free IAA is sandwiched between domain II of the Aux/IAA protein. This enables interaction of the TIR1/AFB auxin receptors with Aux/IAA proteins that shunt Aux/IAAs into the ubiquitin-proteasome pathway. Transcription of the downstream gene occurs once the ARF is depressed by the lability of the Aux/IAA protein, and enhanced by MYB77-ARF interaction with the transcriptional response elements adjacent to the AuxRE. (d) The synthetic auxin-responsive reporter, *DR5rev::eGFP*, consists of multimerized AuxREs within a miminal promoter. This allows the activity of ARF and Aux/IAA proteins to be monitored at the cellular level by observing the output of the eGFP protein.

6.2 Gibberellins

- Gibberellins form a large family of tetracyclic diterpernoid compounds of which only a small number are active PGRs.

- Several lines of evidence have shown that fertilization results in increased levels of GA in the ovary (Eeuwens and Schwabe, 1975; Mapelli *et al.*, 1978; Ozga *et al.*, 1992; van Huizen *et al.*, 1995; Ben-Cheikh *et al.*, 1997; Serrani *et al.*, 2007b, 2008; Vriezen *et al.*, 2007). Due to their high gibberellin content, fertilized ovules have long been considered the source of growth-promoting and fruit-setting compounds.

6.2.1 Gibberellin biosynthesis

In higher plants, GA biosynthesis can be divided into three stages:

- The first stage results in the synthesis of ent-kaurene by the action of two cyclases, ent-copalyl diphosphate synthase (CPS) and ent-kaurene synthase (KS).
- In the second stage, GA12 and/or GA53 are produced as a result of the action of ent-kaurene oxidase (KO) and ent-kaurenoic acid oxidase (KAO) in the case of GA12 and an extra 13-hydroxylation step in the case of GA53.
- The final stage results in the synthesis of active GAs through two parallel pathways (Fig. 4.5):

the non-13-hydoxylation pathway (leading to GA4) and the early 13-hydroxylation pathway (leading to GA1 and GA3 in some cases). Enzymes involved in this final stage include 2-oxoglutarate-dependent dioxygenases, GA 20-oxidases (GA20ox) and GA 3-oxidases (GA3ox).

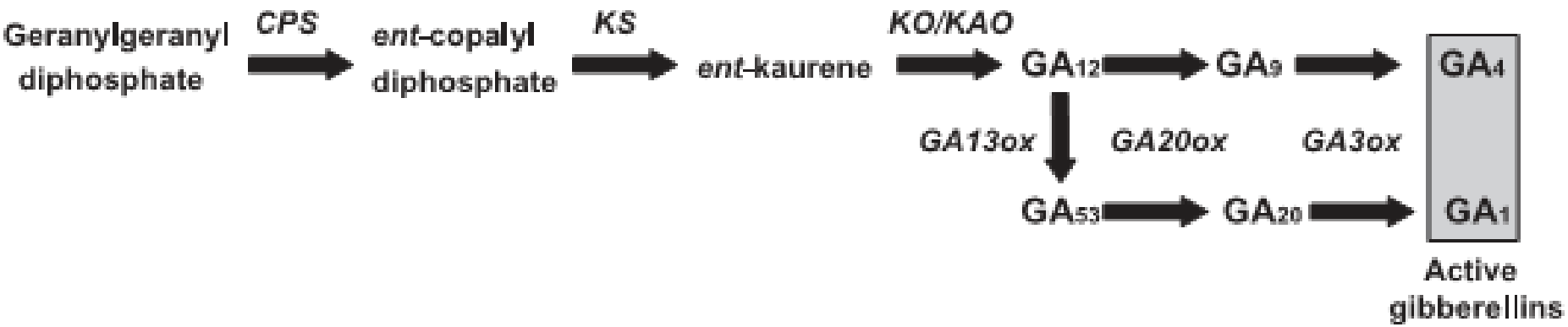


Figure 4.5 The GA biosynthesis pathway in higher plants. The pathway is shown from the common precursor geranylgeranyl diphosphate to the active gibberellins GA_1 and GA_4. The names of the enzymes catalyzing each step are shown in italics.

Our present understanding of the role of GA biosynthesis in fruit initiation and fruit development is based mainly on the study of the final steps of the biosynthetic pathway. Several studies have shown that pollination/fertilization results in increased expression levels of enzymes catalyzing the last steps of GA biosynthesis.

- in pea, removal of seeds reduced the activity of GA 20-oxidase in the pericarp.
- In tomato, pollination/fertilization results in increased expression levels of GA 20-oxidases on the ovary.

2 recent studies:

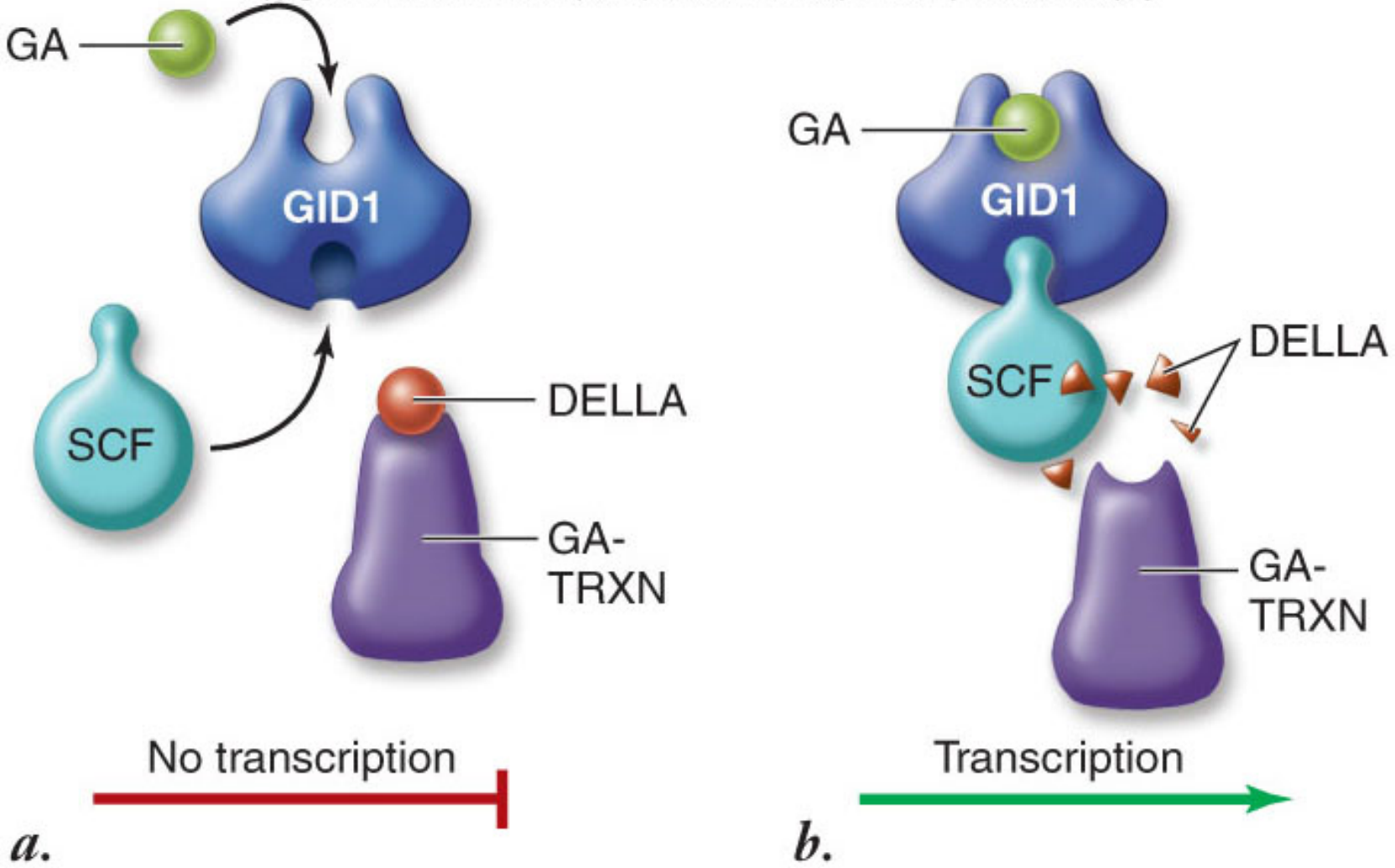
Serrani *et al.* (2007b) showed that while transcript levels of GA 3-oxidases remained constant in both unpollinated and pollinated ovaries, a marked increase in GA 20-oxidase transcript levels was detected upon pollination/fertilization which suggested that fruit initiation in unpollinated tomato ovaries is perhaps partially limited by the low activity of GA 20-oxidases. This is in accordance with the studies carried out in *pat* tomato mutants where the parthenocarpic phenotype can at least partially be explained by the constitutive expression of *GA20ox1* (Fos *et al.*, 2000; Olimpieri *et al.*, 2007). Although in wild-type-pollinated tomatoes as well as in parthenocarpic *pat* tomatoes GA 20-oxidases were found to be expressed throughout the ovary, higher expression levels were observed in the seeds and ovules, respectively (Olimpieri *et al.*, 2007; Serrani *et al.*, 2007b). Therefore, it is possible that seeds (in wild-type plants) and ovules (at least in some parthenocarpy conferring mutations) may be the origin of growth promotion. Similarly, a recent study of *GA3ox* genes in *A. thaliana* has also concluded that developing seeds are likely to be sites of GA biosynthesis (Hu *et al.*, 2008). Analysis of the expression pattern of *GA3ox* genes in developing siliques showed that *GA3ox1* expression is limited to the replum, funiculi and silique receptacle while *GA3ox2*, *GA3ox3* and *GA3ox4* are expressed in developing seeds (Hu *et al.*, 2008). Despite these expression patterns, further mutant analysis concluded that only *GA3ox1* and *GA3ox4* are likely to be involved in the control of fruit initiation and fruit development in *Arabidopsis* (Hu *et al.*, 2008).

6.2.2 Gibberellin signalling

- Similarly to the GA biosynthesis pathway, GA response and signalling path-ways have also been intensively studied in the past few decades and many molecules involved in these pathways have been characterized. However, few components of the GA response and signalling pathways have been studied in the context of fruit initiation and fruit development.

The *SPINDLY* (*SPY*) locus of *Arabidopsis* was one of the first molecular players of the GA signalling pathway shown to be involved in the control of fruit initiation and fruit development (Jacobsen and Oleszewski, 1993). Although doubts still persist about the precise role played by *SPY* in the control of GA-mediated responses, particularly in relation to other components of the signalling pathway (Silverstone *et al.*, 1998), it is generally considered to be a negative regulator of the GA response pathway (Jacobsen and Oleszewski, 1993). During the initial characterization of the SPY locus, it was reported that emasculation of *spy* mutant pistils resulted in parthenocarpic silique elongation. This and other phenotypes of the *spy* mutants were suggested to be the consequence of the constitutive activation of the GA perception and/or GA signal transduction. Nevertheless, attempts to repeat the parthenocarpic silique elongation observed by Jacobsen and Oleszewski (1993) have failed and, thus, the role of SPY in the control of fruit initiation and/or fruit development remains to be clarified (Vivian-Smith *et al.*, 1999).

- **DELLA proteins** are probably the most intensively studied components of the **GA signalling pathway**. They are part of the GRAS transcription factors family and, within this larger family, DELLA proteins are characterized by a conserved N-terminal amino acid sequence which appears to be essential for the regulation of GA responses. It has been shown that DELLA proteins act as growth repressors and GA-mediated degradation of these proteins through the 26S proteosome pathway is required in order to promote growth (Fig. 4.6)



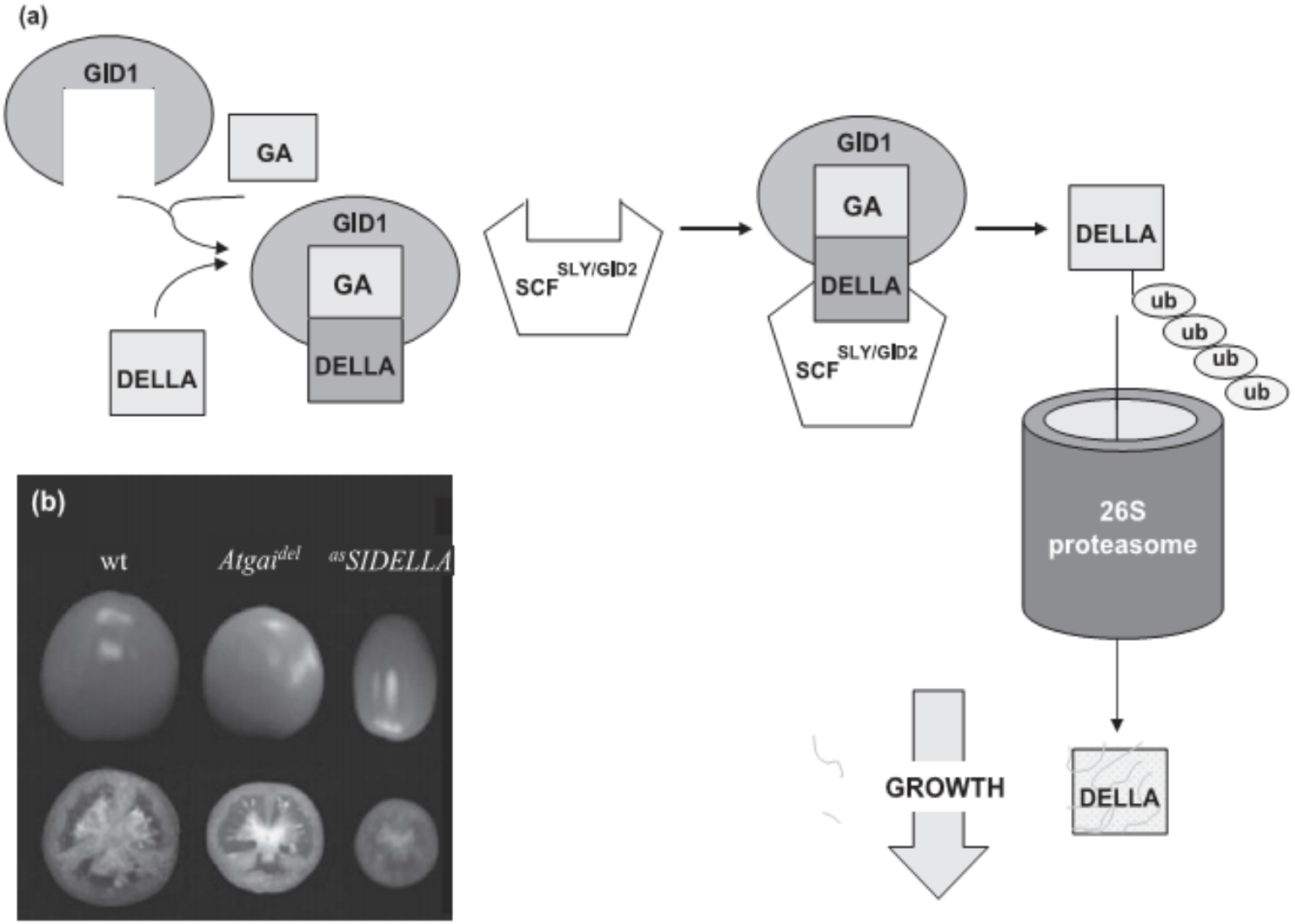


Figure 4.6 (a) Simplified model of GA-mediated DELLA degradation. In the presence of GA, nuclear-localized DELLA proteins associate with the complex form by GA and the GID1 receptor. This association enables the further interaction with the $SCF^{SLY/GID2}$ complex which results in DELLA protein poly-ubiquitination and, ultimately, in growth promotion by the degradation of the DELLA proteins through the 26S proteasome. (b) *DELLA* mRNA silencing causes facultative parthenocarpic fruit development in tomato. From left to right: pollinated wild-type fruit, parthenocarpic *asSlDELLA* transgenic fruit and hand-pollinated *asSlDELLA* fruit (adapted from Marti *et al.*, 2007). Used with permission of the publisher and authors.

6.3 Cytokinin and ethylene perception

- The role of cytokinins in fruit initiation and fruit development has been studied to a considerably lesser degree than other phytohormonal processes.
- Like numerous other phytohormones, cytokinins have also been identified as potential phytohormonal components produced in developing seeds that could promote fruit development.
- Indeed, exogenous cytokinin application to pistils can result in parthenocarpic fruit development in *Arabidopsis*, *Brassica napus* and in pea amongst many other species.
- Additionally, stimulation of cytokinin biosynthesis by using fruit and ovary-specific expression of the *ipt* gene from Agrobacterium produces parthenocarpy in tomato.
- Strong linkage of cytokinins to the control of cell cycle progression has led to the speculation that cytokinins could be responsible for stimulating carpel cell division post-fertilization.

- Cytokinin downregulated *ARF8*, *PIN2* and an auxin biosynthesizing nitrilase gene.
- These three genes are characterized as late responders to cytokinin treatment since the modulation of expression occurred after 120 min.
- This matches well with data that cytokinin treatment to pistils can **trigger an auxin response** in unfertilized Arabidopsis ovules.
- Unfertilized pistils that were treated with benzyl adenine (BA; 1 nmol pistil−1) had a similar auxin response to that observed following pollination (Figs. 4.6b, 4.6c), yet this occurred after a 12 h period.
- This is significantly greater than the auxin treatment described earlier (<2 h) or even that induced by fertilization (<3h). Since the cytokinin-induced auxin response occurs outside these timeframes this suggests **indirect mechanisms of triggering fruit initiation**, or at least that BA treatment had **reduced mobility** when compared to auxin.

- In contrast to cytokinin, **ethylene** has been typically associated with floral and fruit abscission and in fruit ripening.
- Associations of ethylene with fruit initiation have not been intensively investigated, however ethylene precursor molecules, like **1-aminocyclopropane-1-carboxylic acid (ACC**), have been associated with actions that stimulate fruit growth.
- Proof of the definitive involvement of the ACC in fruit initiation: exogenous application of ACC to unpollinated pistils induced fruit elongation (Tang, 2003).
- Genetic analysis proved that ACC induction was completely dependent on AXR1, (a ubiquitin-activating) enzyme involved in the auxin response pathway.
- The genetic analysis revealing the involvement of AXR1 in ACC induced fruit elongation, potentially implicates that the degradation of Aux/IAA proteins is involved in parthenocarpic fruit development triggered by the ACC response.
- These results together with the experiments that show that radio-labelled ACC transport readily occurs in carnation gyneocia may suggest that ACC is an important player in fruit set.

6.4 Hormonal cross-talk

- Treatment of unpollinated ovaries with **auxins, gibberellins or cytokinins** alone does not result in normal fruit development across different plant species and application of specific hormonal combinations is required to trigger fruit development to the extent observed in fully seeded fruit. For example in Arabidopsis, application of gibberellins together with either cytokinins or auxins is required to restore silique length to that of pollinated siliques. These observations suggest that a hormonal interplay is necessary for normal fruit development.

Growing evidence suggests that the stimulation of fruit initiation and growth by seed origin auxin can at least be partially attributed to the upregulation of gibberellin metabolism. van Huizen *et al.* (1995) showed that the conversion of GA_{19} to GA_{20} in pea pericarp is seed regulated and that application of the auxin 4-Chloroindole-3-acetic acid (4-Cl-IAA) can substitute for the seeds in the promotion of this conversion. Similar conclusions were reached by Ngo *et al.* (2002) who found that treatment of deseeded pea pericarps with 4-Cl-IAA increased GA 20-oxidase gene expression. Recent experiments have also shown that auxin-induced parthenocarpy can be blocked by GA-specific inhibitors (mainly paclobutrazol) (Serrani *et al.*, 2008). On the other hand, analysis of the ovary and ovule transcriptomes induced after pollination or by GA_3 treatment provided data that auxin-induced transcripts were unaffected by GA_3 treatment (Vriezen *et al.*, 2007). These results together with the GA-biosynthesis upregulation observed upon auxin treatment (Van Huizen *et al.*, 1995; Ngo *et al.*, 2002; Serrani *et al.*, 2008) and the lost of auxin-induced parthenocarpy upon PCB treatment (Serrani *et al.*, 2008) suggest that auxin stimulation of fruit set is partially mediated by gibberellins while the opposite appears to be improbable.

A recent study has also shown that application of brassinosteroids can induce parthenocarpic cucumber fruit development (Fu et al., 2008). This is in agreement with previous results by Montoya et al. (2005) which showed that Br C-6 oxidase, an enzyme catalyzing what it is thought to be a rate-limiting step in brassinosteroid biosynthesis, is highly expressed in developing seeds in tomato.

Both studies certainly point towards a **role of brassinosteroids in fruit initiation and fruit development.**

Furthermore, brassinosteroids have also been shown to act **synergistically to auxin** in the regulation of several target genes.

For example, the brassinosteroid-regulated BIN2 kinase is able to phosphorylate auxin response factor 2 (ARF2) which results in the loss of ARF2 activity (Vert et al., 2008). In the model proposed by Vert et al. (2008), brassinosteroids release the repression activity of ARFs (such as AFR2) while auxin increases the expression of activator ARFs.

Thus, brassinosteroids and auxin would coregulate gene expression through ARFs activity

A number of studies have also considered the interplay between auxin and other hormones such as cytokinins, ethylene and abscisic acid (ABA) in fruit initiation. Application of cytokinins to unpollinated *Arabidopsis* pistils increased seed origin auxin, suggesting that cytokinin stimulation of fruit initiation is at least partially mediated by auxins (Vivian-Smith and Offringa, unpublished data). On the other hand, analysis of the tomato ovary transcriptome showed that fruit set either by pollination or by gibberellin application resulted in the downregulation of ethylene and ABA biosynthesis (Vriezen *et al.*, 2007). Based on these results, it was concluded that ABA and ethylene might play an antagonistic role to that of auxin and gibberellin in fruit initiation, possibly by keeping the ovaries protected and/or dormant prior to pollination and fertilization (Vriezen *et al.*, 2007).

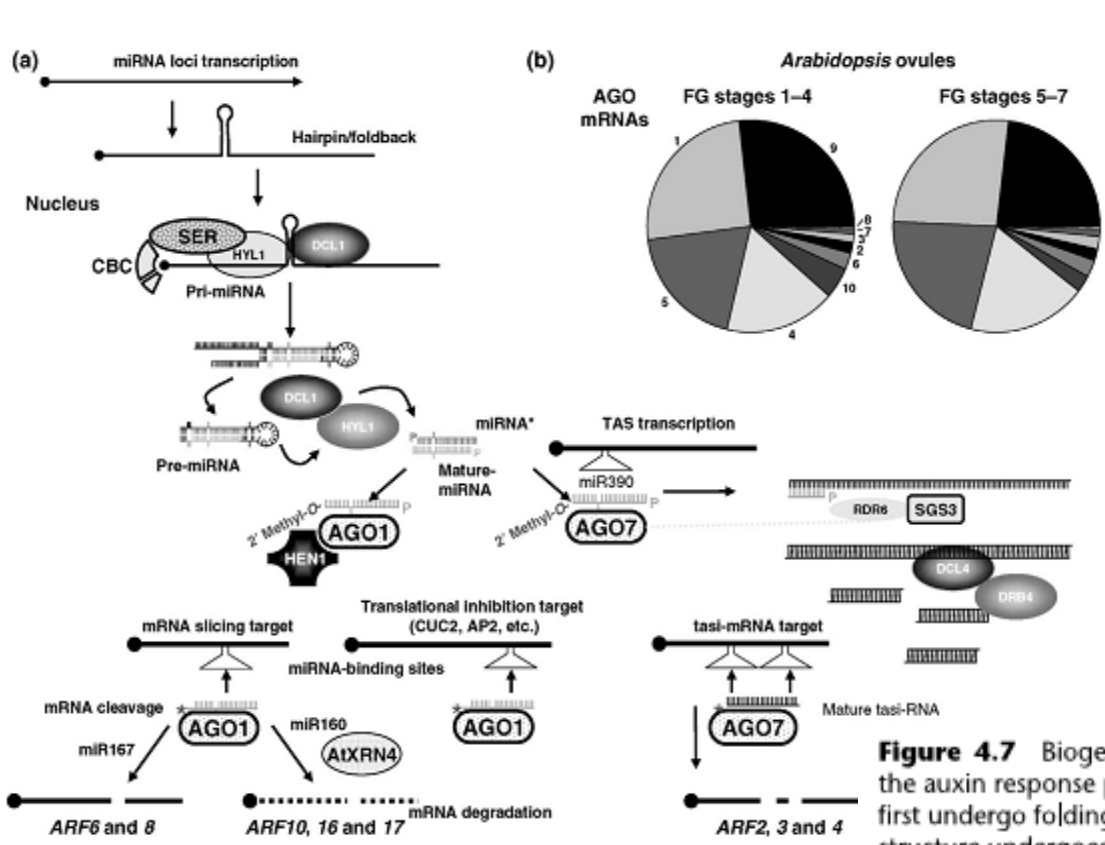


Figure 4.7 Biogenesis of miRNAs and post-translational regulation of mRNA targets in the auxin response pathway. (a) miRNAs are generated from non-coding RNA loci and first undergo folding of primary pre-miRNA (top left). The transcript with hairpin foldback structure undergoes processing by the SER/HYL1/DCL1 complex. The CBC complex binds to the mRNA cap and the mRNA is acted upon by the HYL1/DRB protein and DCL1. This generates a 21 nucleotide double-stranded RNA with 5′ phosphorylated two base overhangs and some internal mismatches. The dsRNA is methylated by HEN1 which adds a 2′-methyl group to the 3′ end, increasing the stability of the miRNA. Double-stranded miRNAs are loaded into AGO1 proteins and the miRNA* strand is lost, thereby creating an active miRNA–AGO complex. AGO1 and AGO10/ZWILLE miRNA RISC complexes identify miRNA targets. Either target cleavage or translational inhibition occurs. The *Arabidopsis ARF6* and *ARF8* mRNAs are miR167 cleavage targets and are degraded by an EIN5/XRN4-independent mediated decay. *ARF10*, *ARF16* and *ARF17* are targeted by miR160 and are degraded by an XRN4-dependent process. The ta-siRNA targets *ARF2*, *ARF3* and *ARF4* are processed by a second order miRNA processing mechanism started by miR390. miR390 is preferentially loaded into AGO7 and targets a TAS3 mRNA. Cleavage generates a phased dsRNA priming site. This is enacted upon by RDR6 and SGS3 to generate long dsRNA which is then processed by the DCL4/DRB4 complex into 21 nucleotide siRNAs. These are loaded into AGO7 and processed to target a variety of mRNAs including *ARF2*, *ARF3* and *ARF4*. (b) Representation of relative levels of AGO mRNA transcripts in ovules at female gametophyte (FG) stages 1–4 (left) and 5–7 (anthesis; adapted from Yu *et al.*, 2005, supplementary data).

8 Signal transduction from ovule to carpel and vascular canalization

- Fruit initiation involves coordinated intra- and inter-organ signalling between the ovule and carpel.

- Numerous signalling components in the ABA, ethylene, cytokinin, GA and auxin pathways are already modulated within 24 h post-pollination.

In a short time period, developmental changes also occur and carbon partitioning is established.

The development of vascular networks in the ovule, carpel and pedicel of the flower facilitates this process.

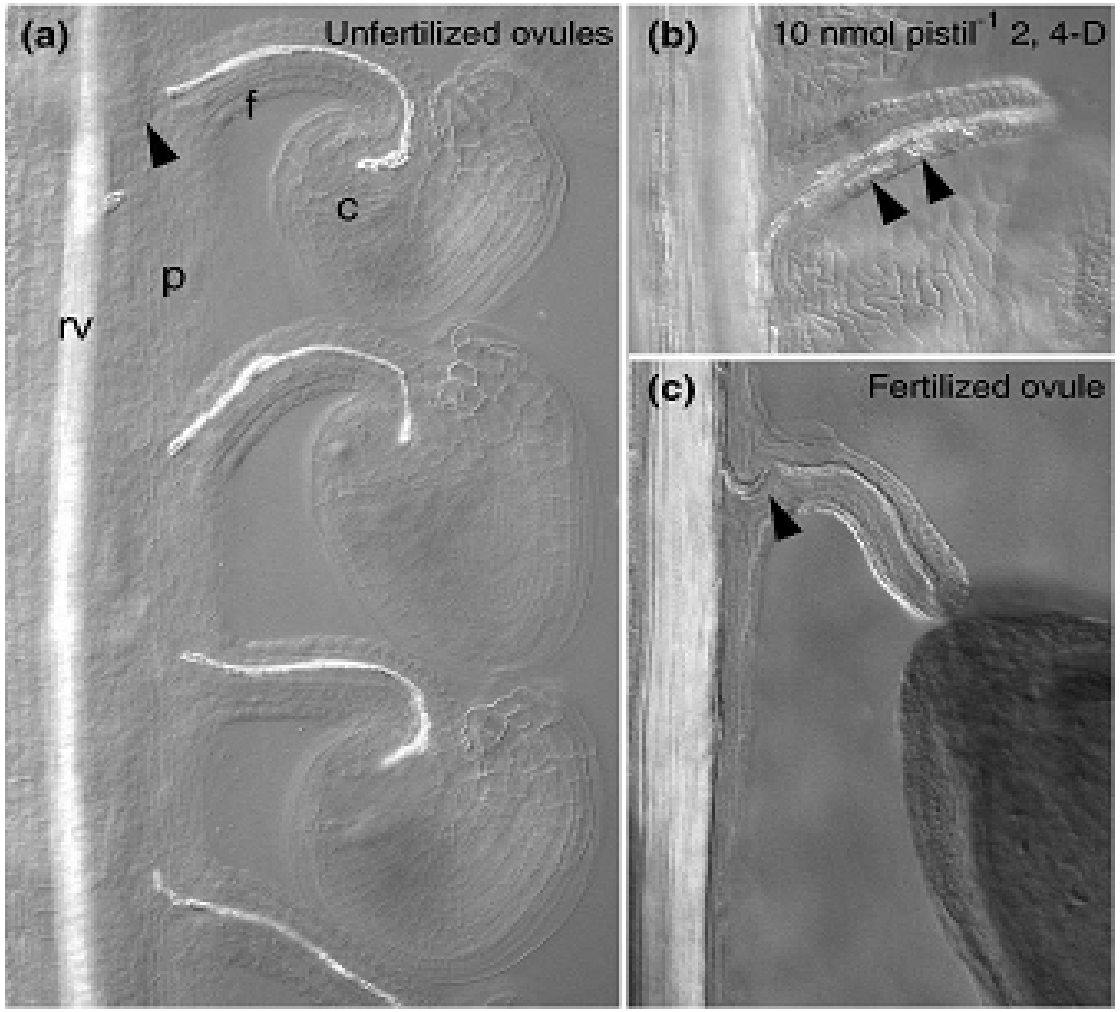


Figure 4.8 Post-fertilization canalization of vascular development in *Arabidopsis* ovules. (a) Development of vascular networks in unfertilized ovules. Unfertilized ovules have a vascular strand that is separated from the replum vascular strand (arrow). (b) Upon treatment with 2–4 D or (c) pollination, vascular biogenesis occurs, thereby joining the replum vascular network with the ovule vascular network. Note that 2–4 D treatment also induces isolated vascular elements throughout the funiculus (arrows). c, chalaza; f, funiculus; p, placental tissue; rv, replum vascular bundle.

Vascular biogenesis and development within the ovule may ultimately alter **carbon partitioning** and **source-sink relationships**.

The processes of vascular biogenesis may not only be restricted to the ovule–carpel vascular junction, but also elsewhere in the flower, since vascular biogenesis, or its absence, in the pedicel has been linked to growth and abscission in citrus, *Prunus* and apple, respectively. Importantly, these observations appear to pinpoint a time when discrimination occurs.

Competition begins between fruit and flowers, and causes the fruit abscission often observed in the first week of many important commercial tree crops. Competition also occurs between parthenocarpic fruit and seeded fruit, which are usually stronger.

Vascular biogenesis presumably reinforces nutrient and photoassimilate allocation to developing fruit affecting retention.

Interestingly, the fruit weight locus (*fw2.2*) from tomato is highly expressed in ovules at anthesis and is responsive to adjacent fruit loads (Baldet *et al.*, 2006). This suggests an early role for *fw2.2* in fruit retention.

9 Current models of fruit initiation

- In leaves, auxin is thought to be synthesized in the marginal tissue and transported away via PAT that is dependent on *MP* and *PIN* efflux carriers.
- Development of provascular strands would act as efficient drainage canals, thereby developing the observed vascular networks.
- Auxin synthesis after fertilization in the integuments, or integument tips, would not only stimulate the formation of the provascular network in the carpel margin, but also the growth of the carpel into the fruit.
- Prior to fertilization, ARF8 is expressed in the female gametophyte, the endothelium of the inner integument and in the chalaza/funiculus regions. At this stage, the activity of ARF8 would restrict the auxin response, possibly through self-reinforcement.

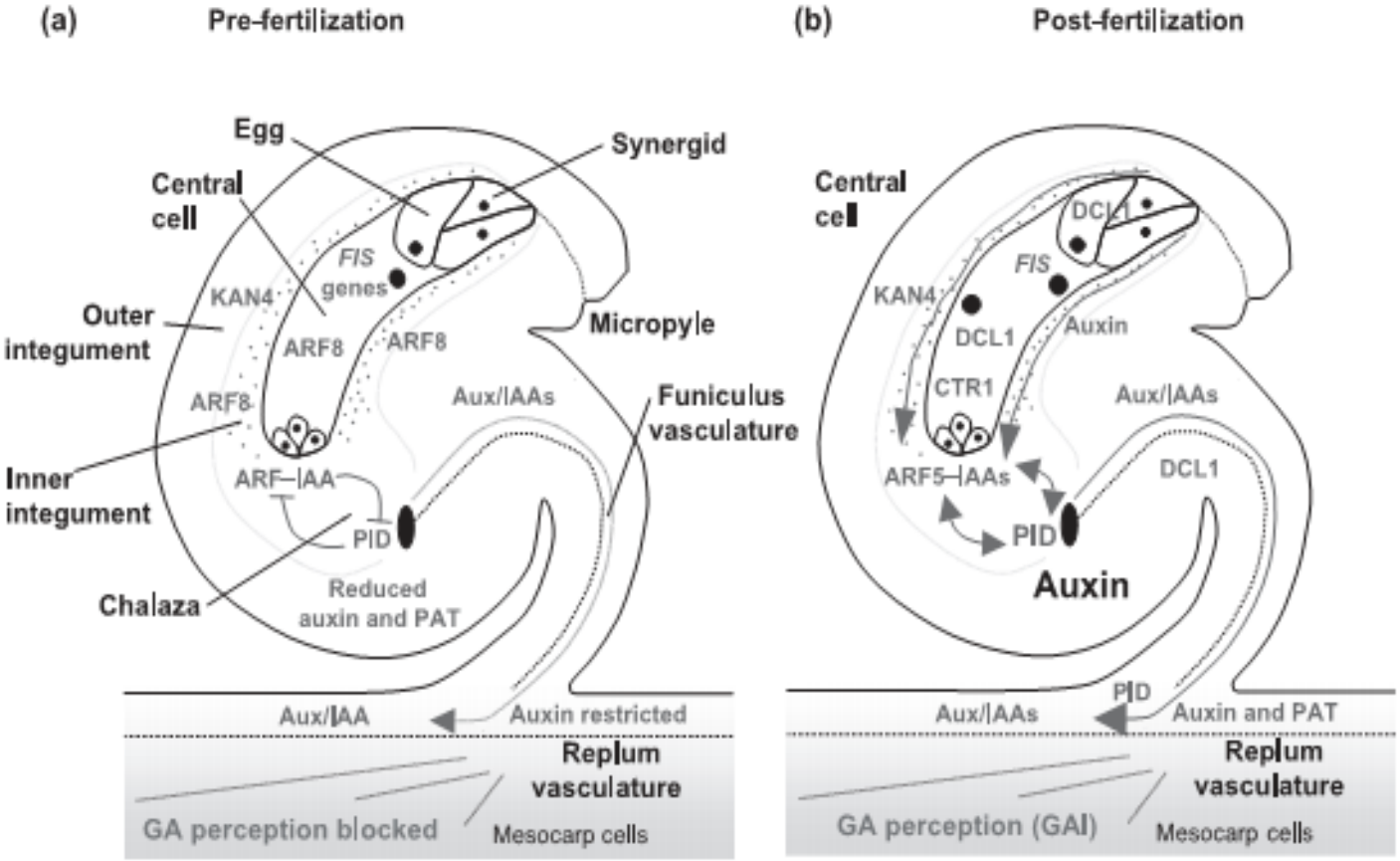


Figure 4.9 Integrated model of fruit initiation. (a) In the absence of fertilization, *FIS* class genes actively restrain central cell growth and autonomous endosperm proliferation. The primary auxin response is also restricted by the activity of ARF8 in the ovules. Specific ovule cells in the pathway shutdown the intracellular auxin response and communication via ARF–ARF and ARF–IAA protein interactions. PID may not play a direct role in this communication but might become important later in the post-fertilization PAT processes. KAN4 may contribute to the synchronization of pistil development before anther dehiscence via control of PAT. In the carpel, GA response remains blocked by the restraint in growth imposed by DELLA proteins. (b) Following double fertilization, zygote and endosperm development is initiated. Concomitant upon the first nuclear division in the endosperm 3–5 h post-fertilization, the primary sporophytic auxin response is initiated in the chalaza and endothelium. The restraint upon auxin response is also eliminated possibly by DCL1-mediated ARF8 removal. Upregulation of the PAT results in the auxin growth response being transmitted to the carpel which in turn, triggers the GA biosynthesis pathway and vascular development. Increased levels of gibberellins cause growth stimulation by DELLA protein degradation.

4.10 Concluding remarks

Many studies have contributed to the better understanding of the complex regulatory system controlling fertilization and fruit initiation. Nevertheless, vital questions remain to be answered. Are hormones the initiators or just systemic components of the signalling cascade? Can we isolate the first step triggering fruit set? What are the signals upstream of phytohormonal signalling that are activated directly after fertilization? Which are the sites of endogenous hormone biosynthesis during fruit initiation and are they regulated in the first steps of fruit initiation? What is the nature of the communication events between the female and male gametophyte? The study of fruit initiation in *Arabidopsis* and other species will undoubtedly help to clarify these and other unknowns.

Finally, a number of publications have stated that *Arabidopsis* and *Brassicaceae* appear to be far from optimal models for fruit development and have suggested that plant species bearing large fleshy fruit offer superior advantages for understanding the molecular basis of fruit initiation. However, genes controlling fruit initiation are likely to be conserved throughout angiosperm plant lineages. Furthermore, many commercial fruit crops have been domesticated over thousands of years and show strong selection for consumer traits (e.g. tomato, Nesbitt and Tanksley, 2002; Bai and Lindhout, 2007; Cong *et al.*, 2008; Xiao *et al.*, 2008; Apple, Harris *et al.*, 2002; *Capsicum*, Paran and Van Der Knaap, 2007; *Phaseolus*, *Curcubitaceae*). This is highlighted by the finding that parthenocarpic figures were intentionally planted as early as 11 200–11 400 years ago in the Jordan Valley (Kislev *et al.*, 2006). As a consequence, many of these crops also show a degree of latent parthenocarpy that does not exist in wild accessions. For example in tomato, 23 commercial cultivars were recently tested all of which displayed certain degree of latent parthenocarpy (Goetz *et al.*, 2007). In contrast, *Arabidopsis* is comparatively free from selected potentiation and latent parthenocarpy and, thus, it is likely to provide a more truthful picture of fruit initiation. Further research together with the transfer of mutant trait loci into crop species will undoubtedly lead to an acceptance of *Arabidopsis* as a tractable model for fruit initiation.