RESEARCH ARTICLE

The Interaction Between DELLA and ARF/IAA Mediates Crosstalk between Gibberellin and Auxin Signaling to Control Fruit Initiation in Tomato

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Short title: GA-auxin crosstalk by DELLA and ARF/IAA

One sentence summary: Gibberellin and auxin regulate tomato fruit initiation through direct crosstalk, a process mediated by a gibberellin signaling repressor and auxin signaling components SlARFs/SlIAA9.

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ABSTRACT

Fruit initiation following fertilization in angiosperms is strictly regulated by phytohormones. In tomato (Solanum lycopersicum), auxin and gibberellin (GA) play central roles in promoting fruit initiation. Without fertilization, elevated GA or auxin signaling can induce parthenocarpy (seedless fruit production). The GA-signaling repressor SIDELLA and auxin-signaling components SIARF7 and SIARF7 repress parthenocarpy, but the underlying mechanism is unknown. Here, we show that SIDELLA and the SIARF7/SIARF9 complex mediate crosstalk between GA and auxin pathways to regulate fruit initiation. Yeast-two-hybrid and co-immunoprecipitation assays showed that SIARF7 and additional activator SIARFs interact with SIDELLA and SIARF9 through distinct domains. SIARF7/SIARF9 and SIDELLA antagonistically modulate the expression of feedback-regulated genes involved in GA and auxin metabolism, whereas SIARF7/SIARF9 and SIDELLA co-regulate the expression of fruit growth-related genes. Analysis of procera (della), SIARF7 RNAi (with downregulated expression of multiple activator SIARFs), and entire (iaa9) single and double mutants indicated that these genes additively affect parthenocarpy, supporting the notion that the SIARFs/SIARF9 and SIDELLA interaction plays an important role in regulating fruit initiation. Analysis of the GA-deficient mutant gib1 showed that active GA biosynthesis and signaling are required for auxin-induced fruit initiation. Our study reveals how direct crosstalk between auxin- and GA-signaling components is critical for tomato fruit initiation.

INTRODUCTION

Fruit development is an important reproductive process in angiosperms (Gillaspy et al., 1993). Fruits are derived from ovaries, whose default fate is senescence. Only upon successful
pollination and fertilization can an ovary develop into a fruit; this process is called fruit initiation or fruit set. In general, fruit development starts with extensive cell division, followed by cell expansion to provide a suitable environment for maturation of the developing seeds inside the fruit (Gillaspy et al., 1993; Ruan et al., 2012). Plant hormones play pivotal roles in fruit development. In particular, auxin and gibberellin (GA) are the major hormones that promote fruit initiation and subsequent growth to enable full-size fruit production (Srivastava and Handa, 2005; Ruan et al., 2012; Seymour et al., 2013). The current model suggests that both auxin and GA are produced in young seeds immediately after fertilization to promote the fruit initiation process. Importantly, the application of either hormone can trigger parthenocarpy (formation of seedless fruit from an unpollinated ovary) in the absence of fertilization, indicating that the activation of auxin or GA signaling is required for fruit initiation (Gorguet et al., 2005). Parthenocarpy is a desirable agronomic trait that uncouples fruit development from the requirement for fertilization, creating seedless fruits that are often preferred by consumers. Auxin and GA are also involved in post-fertilization fruit growth, with distinct functions; in general, auxin promotes cell division and GA functions in later cell expansion (Serrani et al., 2007).

In recent years, the molecular mechanisms of the early auxin and GA signaling cascades have been well characterized, particularly in Arabidopsis thaliana and rice (Oryza sativa); these mechanisms are conserved in angiosperms. Auxin activates its signaling pathway by binding to its nucleus-localized receptors TIR1/AFBs, which are F-box proteins (subunits of an SCF ubiquitin E3 ligase) that recruit downstream repressors AUX/IAA (IAA) proteins for polyubiquitination and subsequent degradation by the 26S proteasome (Dharmasiri et al., 2005; Kepinski and Leyser, 2005; Mockaitis and Estelle, 2008; Salehin et al., 2015). In the absence of auxin, IAA proteins bind to AUXIN RESPONSE FACTOR (ARF) transcription factors to repress auxin signaling. When auxin levels are elevated, auxin induces the rapid degradation of IAA proteins, allowing ARF monomers and/or dimers to activate auxin-responsive genes (Guilfoyle and Hagen, 2007; Guilfoyle, 2015; Salehin et al., 2015). Both IAAs and ARFs belong to large gene families, adding more layers of regulation and complexity to auxin signaling.

Several members of the IAA and ARF families have been shown to function in fruit development. In tomato (Solanum lycopersicum), a model plant for fleshy fruit development, the SlAA9 loss-of-function mutant entire (Zhang et al., 2007) and antisense lines (Wang et al., 2005) showed strong parthenocarpy, indicating its function as a repressor of tomato fruit set.
Downregulation of \textit{SlARF7} by RNAi also confers parthenocarpy (de Jong et al., 2009). In addition, a loss-of-function mutation in Arabidopsis \textit{ARF8} results in a parthenocarpic-fruit phenotype (Goetz et al., 2006). Similarly, downregulation of the eggplant \textit{ARF8} (\textit{SmARF8}) by RNAi causes parthenocarpy (Du et al., 2016). Interestingly, overexpression of \textit{SmARF8} in Arabidopsis also promotes parthenocarpy (Du et al., 2016). These reports suggest that auxin-signaling components interact to regulate fruit set. Similar to auxin signaling, the GA response pathway is negatively regulated by its central repressors DELLA proteins, which are nucleus-localized transcriptional regulators (Peng et al., 1997; Silverstone et al., 1998; Sun, 2010; Daviere and Achard, 2016). Binding of bioactive GA to its nuclear receptor GIBBERELLIN INSENSITIVE DWARF1 (GID1) enhances the GID1-DELLA interaction, resulting in rapid degradation of DELLAs via the ubiquitin-proteasome pathway, thus releasing growth repression imposed by DELLAs (McGinnis et al., 2003; Ueguchi-Tanaka et al., 2005; Murase et al., 2008). Arabidopsis contains five \textit{DELLA} genes: the quadruple mutant (with functional \textit{RGL3} and no other functional \textit{DELLA} genes) and the quintuple \textit{della (global)} mutant show equally strong parthenocarpy, indicating that \textit{RGA, GA INSENSITIVE, RGL1} and \textit{RGL2} play major roles in this process (Dorcey et al., 2009; Fuentes et al., 2012). Similarly, in tomato, mutations and RNAi downregulation of the only \textit{SIDELLA} gene (\textit{PROCERA}) cause parthenocarpy (Marti et al., 2007; Carrera et al., 2012; Livne et al., 2015), confirming that \textit{PROCERA} is a repressor of fruit initiation.

The elucidation of auxin and GA signaling cascades has greatly facilitated investigations of how these hormones coordinate with each other to regulate fruit development. The current model (based on studies in several organisms) suggests that auxin acts upstream of GA during fruit development (Serrani et al., 2008; Dorcey et al., 2009; Ozga et al., 2009). In Arabidopsis, the fertilization-induced auxin response or auxin application triggers GA biosynthesis, whereas GA application does not induce the auxin response (Dorcey et al., 2009). In addition, auxin treatment of the \textit{della global} mutant does not further stimulate its parthenocarpy, implying that in Arabidopsis, auxin-induced parthenocarpy occurs entirely through DELLA-dependent GA signaling (Fuentes et al., 2012). In tomato, parthenocarpic induction by auxin is also mediated at least partially by GA, as co-treatment with paclobutrazol (PAC, an inhibitor of GA biosynthesis) and auxin greatly reduces parthenocarpy, which can be reversed by co-application with GA (Serrani et al., 2008). In addition, bioactive GA levels are elevated in auxin (treatment or
iaa9/entire)-induced parthenocarpic fruits due to increased expression of GA biosynthesis genes and reduced expression of GA catabolism genes (Serrani et al., 2008; Mignolli et al., 2015). Therefore, it was suggested that auxin induces fruit initiation in part through the induction of GA biosynthesis. Auxin and GA are thought to play distinct roles in post-initiation fruit growth, because auxin- and GA-induced parthenocarpic fruits have different morphologies (Serrani et al., 2007). Auxin increases fruit size by increasing the number of pericarp cell layers and enlarging the placenta. GA treatment only induces the formation of medium-size fruit with larger cells but fewer cell layers. Simultaneous application of both hormones can promote parthenocarpy, yielding fruit with a size and cellular structure similar to those of pollinated fruit. Overall, these studies point to a possible mechanism in which auxin acts upstream of GA during fruit initiation, while these two hormones might play diverse roles during fruit growth.

In addition to the current model of auxin acting upstream of GA, evidence suggesting direct crosstalk between auxin and GA signaling pathways has emerged in recent years. RGA (a major Arabidopsis DELLA) was recently shown to interact with three activator ARFs (AtARF6, AtARF7 and AtARF8) by yeast two-hybrid (Y2H) assays and co-immunoprecipitation (co-IP) (Oh et al., 2014). Through this interaction, RGA appears to inhibit the binding of AtARF6 to the promoters of its target genes that function in hypocotyl elongation (Oh et al., 2014). However, the effect of the DELLA-ARF interaction in planta has not been confirmed by genetic analysis.

In this study, we report that GA and auxin regulate tomato fruit initiation through mediating crosstalk between SlDELLA and SlARF7/SI AA9. Y2H and co-IP assays revealed that SlARF7 and additional activator SIARFs bind to SlDELLA and SI AA9 through distinct domains. RT-qPCR and transient expression analyses showed that the SlARF7/SI AA9 complex and SlDELLA antagonistically regulate the expression of feedback-regulated genes involved in auxin and GA metabolism. By contrast, the SlARF7/SI AA9 complex and SlDELLA additively co-regulate the expression of downstream growth-related genes. To investigate the effect of the DELLA-ARF7/IAA9 interaction in planta, we generated double mutants using procera, SlARF7 RNAi, and entire (iaa9) mutants. Phenotype and expression analysis indicated that these mutations additively affect parthenocarpic fruit development. Moreover, auxin application and arf7/iaa9 mutations failed to promote parthenocarpy in the GA-deficient gib1 mutant, indicating that active GA biosynthesis and signaling are required for auxin-induced fruit initiation. Genetic analysis of slarf7 and slarf5 single and double mutants showed that the parthenocarpic
phenotype of the *SLARF7 RNAi* line is caused by down-regulation of multiple activator *SLARFs*. Overall, our study reveals a mechanism in which direct crosstalk between auxin and GA signaling controls tomato fruit initiation.

**RESULTS**

**GA signaling repressor SIDELLA interacts with auxin signaling transcription factors**

Previous studies have shown that both SIARF7 and SIDELLA affect tomato fruit initiation (Marti et al., 2007; de Jong et al., 2009). We hypothesized that SIARF7 and SIDELLA mediate crosstalk between GA and auxin signaling during fruit development. To test whether SIARF7 and SIDELLA directly interact, we performed a Y2H assay. A truncated SIDELLA construct (DELLA-CT3, encoding the C-terminal portion of SIDELLA from amino acid residues 166-588) was used as the bait because full-length SIDELLA alone can activate the reporter genes in Y2H assays. We found that SIDELLA indeed interacted with SIARF7 in this assay (Figure 1A). We then tested whether this interaction occurs in plant cells by co-IP using the transient expression system in wild tobacco (*Nicotiana benthamiana*). HA-SIDELLA was transiently expressed alone, or co-expressed with Myc-SIARF7 or Myc-GUS-NLS (as a negative control). After IP using anti-Myc antibody-conjugated beads, HA-SIDELLA was co-immunoprecipitated only when co-expressed with Myc-SIARF7, but not with Myc-GUS or when expressed alone (Figure 1B), thus confirming that SIDELLA and SIARF7 interact *in planta*. ARFs, especially the activator subgroup, are known to interact with IAA proteins through their C-terminal PB1 (Phox and Bem1) domains (Vernoux et al., 2011). SIARF7 belongs to the activator subgroup of ARFs. Therefore, we examined whether SIARF7 interacts with SIIAA9, which has been shown to inhibit fruit initiation (Wang et al., 2005). As shown in Figure 1C, SIARF7 interacted strongly with SIIAA9 in a Y2H assay, whereas SIDELLA did not. Furthermore, we performed a yeast three-hybrid (Y3H) assay to test whether SIDELLA and SIIAA9 compete for binding to SIARF7. Neither SIDELLA nor SIIAA9 interfered with the SIARF7-SIIAA9 or SIARF7-SIDELLA interaction, respectively (Figure 1D). These results suggest that SIDELLA and SIIAA9 interact with different regions of SIARF7. To verify this notion, we identified DELLA-interaction domain in SIARF7 using a series of SIARF7 truncation constructs in Y2H assays. As shown in Figures 1E-1F, amino acid residues 375-492 of SIARF7
(ARF7-MR2) are responsible for interacting with DELLA. This region is highly variable among ARFs and is mostly located within the activator/repressor domains (Guilfoyle and Hagen, 2007; Chapman and Estelle, 2009). As expected, the conserved C-terminal PB1 domain of ARF7 (in ARF7-CT3, amino acids 1000-1137) is responsible for binding to IAA9 (Figures 1E-1F). A recent report revealed a second dimerization domain (DD) in ARFs for ARF homodimer formation; this domain flanks the N-terminal DNA binding (DBD) domain (Boer et al., 2014). Interestingly, the SlDELLA-interaction domain in SIARF7 is near this N-terminal ARF dimerization domain (Figure 1E). We therefore tested whether SlDELLA interferes with ARF7 self-dimerization by Y3H assays, using an ARF7-NT1 construct as the bait. Consistent with previous findings (Boer et al., 2014), SIARF7 self-dimerized through its N-terminal region (Figure 1G). However, neither SlDELLA nor SlIAA9 affected SIARF7 self-dimerization in this assay. These results indicate that SlDELLA and SlIAA9 interact with SIARF7 through distinct regions.

Besides SIARF7, the tomato genome contains seven additional activator ARF genes. However, the available SIARF6A sequence only contains a partial coding sequence, and SIARF6B is predicted to encode a truncated protein (Zouine et al., 2014). We tested the five remaining SIARFs (i.e., SIARF5, SIARF8A, SIARF8B, SIARF19A, SIARF19B) by Y2H assays and showed that all of them interacted with SlDELLA and SlIAA9 (Figure 2A). We adopted the same SIARF gene names listed in Zouine et al. (2014), with the exception of SIARF7 and SIARF19A/19B, which were designated SIARF19 and SIARF7A/7B, respectively, in this 2014 report. This is because earlier publications by de Jong et al. (2009 and 2011) already used SIARF7 for Solyc07g042260.

As described above, neither SlDELLA nor SlIAA9 affected SIARF7 homo-dimerization in the Y3H assays. Based on sequence homology of their DD domains, ARFs were proposed to form heterodimers as well (Boer et al., 2014). We tested whether SlDELLA or SlIAA9 would interfere with ARF heterodimer formation. In a Y3H assay, we found that SIARF7-NT1 formed heterodimers with SIARF8A, SIARF8B and SIARF19B, respectively. However, co-expression of SlDELLA or SlIAA9 had little effect on these interactions (Supplemental Figure 1).

Downregulation of multiple activator SIARF genes leads to a pathenocarpic phenotype in the SIARF7 RNAi line
Absolute RT-qPCR analysis showed that all activator *SLARFs* were expressed in ovaries around anthesis and that *SLARF5*, *SLARF8A* and *SLARF8B* were expressed at higher levels than *SLARF7* (Figure 2B and Supplemental Figure 2). However, only *SLARF5* displayed similar temporal expression patterns to those of *SLARF7*: Both *SLARF5* and *SLARF7* were induced at anthesis (0 day after anthesis, or 0 DAA) and down-regulated at 3 DAA. We tested whether any of these *SLARFs* (besides *SLARF7*) are down-regulated in a previously generated *SLARF7 RNAi* transgenic line (de Jong et al., 2009). As shown in Figure 2C, the mRNA levels of *SLARF5*, *SLARF7* and *SLARF8B* in 0 DAA ovaries were reduced 40-60% in the *SLARF7 RNAi* line compared to WT, whereas the expression of the other activator *SLARFs* was not significantly altered. We also analyzed the expression of nine repressor *SLARFs* (*ARF1, ARF2A, ARF2B, ARF3, ARF4, ARF9A, ARF10A, ARF16A, ARF24*) that are expressed in 0 DAA WT ovaries (http://tomexpress.toulouse.inra.fr/). None of these repressor *SLARFs* showed reduced expression in the *SLARF7 RNAi* line compared to the WT (Figure 2D). Taken together, the fruit development phenotypes observed in this *SLARF7 RNAi* line are likely caused by the down-regulation of *SLARF7*, *SLARF5* and *SLARF8B*.

To verify the idea that parthenocarpy in the *SLARF7 RNAi* line is caused by the simultaneous downregulation of multiple activator *SLARFs*, we generated a *SLARF7* null mutant (*CR-slarf7*) by CRISPR-Cas9, as well as a *SLARF5* null mutant (*slarf5-1*, also named *slmf-1* because *SLARF5* is most similar to *AtARF5=AtMP*) in the M82 cultivar background. The *CR-slarf7* allele contains a 107-bp deletion (nucleotides 92-198 downstream of the translational start site) in *SLARF7* that leads to an early stop codon after nucleotide 99. The *slarf5-1* allele was identified in an ethyl methane sulfonate population. This mutant contains a C-to-T (Gln-to-Stop) substitution at nucleotide 754 of the open reading frame downstream of the translational start site, within exon 8. As the *slarf5-1* mutant displayed severe defects in flower development, only the heterozygous mutant (*slarf5/+) was used to test the parthenocarpic phenotype. We also generated a double mutant that is homozygous for *CR-slarf7* and heterozygous for *slarf5-1* (*arf7 arf5/+*) by genetic crosses. The single mutants *slarf7* and *slarf5/+* and the double mutant *arf7 arf5/+* showed normal fruit growth compared to the WT (Figure 2E). Neither single mutant produced parthenocarpic fruits from emasculated flowers, whereas the double mutant *arf7 arf5/+* developed parthenocarpy after emasculation (Figures 2E-2G), indicating that both *SLARF5* and *SLARF7* are involved in fruit initiation. However, the *CR-slarf7 slarf5/+* double mutant produced
parthenocarpic fruits at a lower frequency than the *ARF7 RNAi* line. These results suggest that the parthenocarpic phenotype of the *SlARF7 RNAi* line is caused by down-regulation of multiple activator *SlARFs*. The *SlARF7 RNAi* line, with its clear parthenocarpic phenotype, represents a valuable tool for studying the role of the *SIDELLA-SlARF* interaction in fruit set.

**SIDELLA and SlARF7/SlIAA9 antagonistically regulate the expression of GA/auxin feedback-regulated genes**

Our finding that *SIDELLA* directly interacts with the activator *SlARFs* suggests that these key regulatory proteins may coordinate GA and auxin signaling activity during fruit initiation and development. In the rest of this report, we mainly focused on the interaction between *SlARF7* and *SIDELLA*. To investigate whether and how the *SIDELLA-SlARF7* interaction controls the expression of common target genes, we analyzed the transcript levels of early GA-responsive genes (*SlGA20ox1*, *SlGA3ox1*; Serrani et al., 2008) and auxin-responsive genes (*SlGH3.2*; Kumar et al., 2012) in ovaries of WT tomato flowers at −1 DAA. These genes were chosen because they are likely the direct targets of *SIDELLA* and/or *SlARFs*. The use of −1 DAA ovaries allowed us to examine the effects of auxin and GA on the expression of these genes. Flower morphology around anthesis is shown in Figure 3A. *SlGA20ox1*, *SlGA3ox1* and *SlGH3.2* were repressed by GA application compared to mock treatment (Figure 3B). By contrast, treatment with 2,4-D (a synthetic auxin analog) induced the expression of all three genes (Figure 3C). To test whether *GA20ox1*, *GA3ox1* and *GH3.2* are common targets of *DELLA/ARF7-IAA9* during early GA/auxin signaling, we examined the expression of these genes in loss-of-function or reduced expression mutants of *SIDELLA*, *SlARF7* and *SlIAA9*. Consistently, *GA20ox1*, *GA3ox1* and *GH3.2* expression was reduced in *procera* (*pro*, a *SIDELLA* loss-of-function mutant) but increased in the *SlARF7 RNAi* line and *entire* (*SlIAA9* loss-of-function mutant) compared to WT (Figures 3D-3E), indicating that *SIDELLA* activates the expression of these genes while *SlARF7* and *SlIAA9* repress their expression. *GH3.2* belongs to group II of the GH3 protein family; some group II members in Arabidopsis and rice are auxin-amino acid conjugating enzymes that convert auxin to an inactivate form (Staswick et al., 2005; Park et al., 2007). Thus, our result suggests that GA may regulate auxin homeostasis through *GH3.2*. However, some members of the GH3 family conjugate amino acids to jasmonic acid or salicylic acid instead of auxin (Staswick et al., 2002). To investigate whether auxin can serve as
a substrate for GH3.2, we performed an in vitro enzyme assay using GST-GH3.2 protein produced in *E. coli*. As shown in Supplemental Figure 3, GST-GH3.2 conjugated aspartic acid to IAA, but GST alone did not, suggesting that GH3.2 is an auxin-amino acid conjugating enzyme.

Because the transcript levels of *SlGA20ox1*, *SlGA3ox1* and *SlGH3.2* in –1 DAA ovaries were reduced in *procera* compared to WT and increased in *SIARF7 RNAi* and entire (Figures 3D-3E), we hypothesized that the SIARF7-SIIAA9 complex represses the transcription of *SlGA20ox1*, *SlGA3ox1* and *SlGH3.2*, whereas SIDEELLA induces the expression of these genes by interacting with SIARF7. In the absence of SIIAA9, SIARF7 may act as a transcriptional activator either as a homodimer or heterodimer with other ARFs, as it contains a glutamine-rich sequence and is predicted to be a transcriptional activator (de Jong et al., 2009). To test this possibility, SIDEELLA, SIARF7 and SIIAA9 were transiently expressed alone or co-expressed in *N. benthamiana* leaves by agroinfiltration to determine whether they compete to modulate the transcription of *SlGA20ox1*, *SlGA3ox1* and *SlGH3.2* using a dual luciferase (LUC) reporter assay. The reporter constructs contained promoter sequences of *GA20ox1*, *GA3ox1* and *GH3.2*, which were fused to the firefly LUC gene (*fLUC*). The *Pro35S:Renilla LUC* (*rLUC*) construct was used as an internal standard. Three effectors, *Pro35S:SIDEELLA*, *Pro35S:SIARF7* and *Pro35S:SIIAA9*, were included in the assays. As shown in Figures 3F-3H, SIDEELLA alone induced transcription of these target genes, while SIIAA9 repressed their transcription, which is consistent with the above *in vivo* expression data. SIARF7 alone did not alter the expression of these three genes. However, when combined with SIIAA9, SIARF7 caused further repression of the target genes compared to SIIAA9 alone (Figures 3F-3H), suggesting that SIARF7 may recruit SIIAA9 to these promoters for transcriptional repression. Co-expression of SIDEELLA with SIARF7 and SIIAA9 resulted in intermediate *fLUC* activity (lower than that in the SIDEELLA alone sample, but higher than the SIARF7/SIIAA9 sample). These results strongly suggest that through an interaction with SIARF7, SIDEELLA antagonizes the repressive effect of SIARF7/SIIAA9 on these target genes, which are under feedback regulation and are important for GA and auxin homeostasis.

We then carried out ChIP-qPCR to determine whether SIDEELLA and SIARF7 bind directly to the promoters of these feedback-regulated target genes in vivo. For ChIP of SIDEELLA-binding sites, we generated a transgenic tomato line containing *ProSIDEELLA:FLAG-SIDEELLA* in the *procera* mutant background. In this line, the FLAG-SIDEELLA fusion protein is
functional as it rescued the overall phenotypes of *procera*, including stem growth, leaf shape and fruit development (Supplemental Figures 4A-4D). In addition, FLAG-SIDELLA is responsive to GA-induced degradation (Supplemental Figure 4E). Using −1 DAA ovaries from the FLAG-SIDELLA line, crosslinked chromatin was pulled down using anti-FLAG beads, and qPCR was performed on several primer pairs that span the promoter region of each feedback-regulated target gene (Figure 4A). *SlUBQ7* was used to normalize the qPCR results in each ChIP sample. A 1.8- to 2.8-fold enrichment was observed for promoter sequences of *GA20ox1*, *GA3ox1* and *GH3.2* (Figure 4B). To test whether SIARF7 also binds to these promoters, we transiently expressed a Myc-SIARF7 construct in developing tomato fruits by agroinfiltration because the stable transgenic line was not available. When we performed ChIP using anti-Myc beads, and a 2.8- to 3.7-fold enrichment of promoter sequences of these target genes was observed by qPCR (Figure 4C). These results indicate that both SIDELLA and SIARF7 directly associate with the promoters of these target genes.

To examine whether SIDELLA interferes with the binding of SIARF7 to the promoters of these feedback-regulated genes, we pretreated WT plants with PAC for two weeks and transiently expressed Myc-SIARF7 in developing fruits, followed by GA or mock-treatment. Immunoblot analysis indicated that SIDELLA protein levels were enhanced by PAC and that GA treatment dramatically reduced SIDELLA protein levels (Supplemental Figure 5). ChIP-qPCR showed that GA treatment slightly increased SIARF7 binding to *GA20ox1* and *GH3.2* promoters compared to the mock-treated control (Figure 4D). These results suggest that the SIDELLA-SIARF7 interaction inhibits the binding of SIARF7 to these target promoters. On the other hand, when transiently expressed in fruits of WT or *SIARF7 RNAi*, Myc-DELLA protein bound to its feedback-regulated target genes to similar levels in these plants, as shown by ChIP-qPCR (Figure 4E). These results suggest that the reduced SIARF7 level in *SIARF7 RNAi* had no obvious effect on the association of DELLA with its target promoters.

**SIDELLA and SIARF7/SIIAA9 co-regulate downstream target genes in developing fruits**

Although SIDELLA and SIARF7/SIIAA9 play opposite roles in modulating the expression of feedback-regulated genes involved in GA and auxin metabolism, mutations in *SIIAA9* and *SIDELLA*, as well as the downregulation of *SIARF7* together with additional related ARF genes, lead to fertilization-independent fruit initiation. Therefore, it is possible that
SIDElla and SlARF7/SlIAA9 co-regulate common downstream target genes that control fruit initiation in response to GA and auxin signals. Tang et al. (2015) performed transcriptome profiling of GA- and auxin-induced parthenocarpic tomato fruit at 4 days after hormone treatments. Importantly, the expression of a number of genes is regulated similarly by both hormones, making them candidates for early fruit development genes that are co-regulated by SIDElla and SlARF7/SlIAA9. To test this idea, we analyzed the expression of 14 selected genes in –1 DAA WT ovaries to determine whether they are co-regulated by GA and auxin at 6 and 24 h after treatment (Supplemental Figure 6). Among these, 10 genes, including EXPANSIN5 (EXP5, Solyc02g088100) and ACC OXIDASE4 (ACO4, Solyc02g081190), were co-regulated by GA and auxin at these early time points. EXP5 likely promotes cell expansion during fruit development. ACO4 is an ethylene biosynthesis gene, which may regulate fruit set because ethylene inhibits this process (Vriezen et al., 2008; Shinozaki et al., 2015). Figure 5A shows that EXP5 was induced by 6 hr of GA and auxin treatment, while ACO4 was repressed by both hormones. Consistently, EXP5 was up-regulated in procera, ARF7 RNAi, and entire mutants, while ACO4 was down-regulated in all three lines (Figure 5B). Therefore, SIDElla and SlARF7/SlIAA9 have similar effects on EXP5 and ACO4 expression. ChIP-qPCR analysis showed that both SIDElla and SlARF7 are associated with the promoters of these two genes in vivo (Figures 5C-5E), indicating that EXP5 and ACO4 are direct targets of SIDElla and SlARF7. We then tested whether DELLA levels affect the binding of SlARF7 to these target promoters by ChIP-qPCR analysis. WT plants were pretreated with PAC before Myc-SlARF7 was transiently expressed in developing fruits, followed by GA or mock-treatment. ChIP-qPCR analysis showed that SlARF7 displayed slightly reduced binding to the EXP5 and ACO4 promoters in GA-treated sample (Figure 5G), suggesting that the SlARF7-SIDElla interaction may enhance SlARF7 binding to these target promoters. By contrast, the association of Myc-SIDElla with these promoters was not affected by reduced levels of activator SlARFs in the SlARF7 RNAi lines compared to that in WT (Figure 5F). Thus the association of SIDElla with these promoters does not depend on SlARF7.

Additive interactions among procera, SlARF7 RNAi (downregulation of multiple activator SIARFs), and entire (iaa9) mutations during parthenocarpic fruit development.
To further investigate the effects of the SlDELLA and SlARFs/SlIAA9 interaction on fruit set and growth, we generated double homozygous mutants by genetic crosses using procera, SlARF7 RNAi, and entire mutant lines. The SlARF7 RNAi line was used in this genetic analysis because it displays a clear parthenocarpic-fruit phenotype due to the downregulation of several activator SIARFs (SlARF5, SlARF7 and possibly SlARF8B). We compared the fruit phenotypes of these single and double mutants, including fruits produced after self-pollination and parthenocarpic fruits from emasculated flowers, to those of the single mutants and wild-type (WT) plants. Because procera and entire are in the Ailsa Craig (AC) background, while the SlARF7 RNAi line is in the Moneymaker (MM) background, we included both the AC and MM cultivars and the hybrid AC/MM (F1 of AC x MM) as WT controls in these experiments. The entire (e) and procera (pro) mutations in the e pro double mutant displayed additive effects in terms of parthenocarpic fruit development compared to entire and procera single mutants (Figures 6A-6B). The parthenocarpic fruits (from emasculated flowers) of the e pro double mutant had more advanced fruit structure compared to the other lines, including further developed placenta, locule tissues, and pseudoembryos (Figures 6A-6B), as well as larger fruit size (Figure 6C). By contrast, SlARF7 RNAi procera (ARF7i pro) and entire SlARF7 RNAi (e ARF7i) double mutants mainly showed additive effects in terms of growth in the placenta and locule tissues (Figures 6A-6B), with unaltered fruit size (Figure 6C). Nevertheless, when we measured the efficiency of parthenocarpy formation (i.e., percentage of parthenocarpic fruits that formed vs. total ovaries emasculated), all three double mutants showed significantly higher efficiency than their corresponding single mutants (Figure 6D). These results, together with our biochemical data, support the notion that crosstalk between GA and auxin occurs during fruit initiation through interactions among SIDELLA, SIARFs and SlIAA9. Furthermore, we analyzed the expression of their co-regulated downstream target genes in the double mutants. In e pro, EXP5 and ACO4 were more highly induced or repressed, respectively, than those in the single mutants (Figures 6E-6F). Overall, the results from double mutant phenotyping and target gene analysis support the idea that the interaction of SIDELLA with SIARFs/SlIAA9 has an important impact on their downstream target genes, as well as fruit set and subsequent growth.

Active GA biosynthesis and signaling is a prerequisite for tomato fruit initiation
A previous study suggested that auxin induces parthenocarpy in tomato via GA-dependent and -independent pathways (Serrani et al., 2008). This model is based on the observation that treatment with the GA biosynthesis inhibitor, PAC, did not block auxin-induced tomato fruit initiation, but it only partially inhibited parthenocarpic fruit growth (Serrani et al., 2008). However, depending on the treatment conditions, PAC treatment may not completely block GA biosynthesis and could have non-specific effects. To verify the current model, we used the severe GA-deficient tomato mutant gib1 to test the effect of auxin on parthenocarpy. The gib1 mutant is impaired in the first committed step in GA biosynthesis, which is catalyzed by ent-copalyl diphosphate synthase (CPS) (Bensen and Zeevaart, 1990). GIB1 has been mapped to chromosome 6 (Koornneef et al., 1990). However, the molecular lesion in the gib1 mutant has not been reported. By performing BLAST searches using the AtCPS protein sequence as a query, we identified Solyc06g084240 as a putative SlCPS. This locus is indeed labeled as CPS1 in the Sol Genomics Network website (https://solgenomics.net/). DNA sequence analysis showed that the SlCPS cDNA from the gib1 mutant contains a deletion of G at nucleotide 563 (from the ATG start site), which leads to four new amino acids after amino acid 187, followed by a premature stop codon. Further sequencing of the nearby genomic region in gib1 revealed that instead of a deletion, there is a G-to-A mutation at the end of intron 4 (nucleotide 1178 from the start codon). This mutation shifted the intron excision site one nucleotide into exon 5 (from TA\text{AG}|GAA to TA\text{AG}|AA, where | indicates a splicing site). Taken together, we confirmed that gib1 is a loss-of-function allele of SlCPS (Solyc06g084240).

Consistent with previous studies (Serrani et al., 2007; Serrani et al., 2008), in WT plants, GA or auxin treatment of emasculated −1 DAA ovaries promoted seedless fruit growth (Figure 7A). To understand the role of GA in these responses, we tested how −1 DAA gib1 ovaries respond to GA and 2,4-D treatment after emasculation. As shown in Figure 7B-7C, three weeks after auxin treatment, gib1 ovaries were the same size as mock-treated ovaries. By contrast, GA treatment dramatically promoted parthenocarpic fruit development in gib1. Cross-sections of mock- vs. auxin-treated gib1 ovaries showed similar pericarp and placenta structures, further supporting the observation that auxin failed to promote seedless fruit set in gib1 (Figure 7D). To rule out the possibility that auxin treatment of gib1 ovaries was not effective, we generated ARF7 RNAi gib1 (ARF7i gib1) and entire gib1 (e gib1) homozygous double mutants by crosses to test
whether enhanced auxin signaling by *ARF7 RNAi* or *entire* can promote parthenocarpy in *gib1*. Emasculation experiments showed no parthenocarpy in these double mutants compared to the *gib1* single mutant (Figures 7E-7F). *e gib1* consistently produced smaller ovaries than *gib1* and *ARF7i gib1*, likely due to the pleiotropic effects of *entire*. Analysis of cross-sections of the double mutant ovaries further confirmed that there was no difference in the number of cell layers or cell size compared to the *gib1* single mutant (Figures 7D and 7G). These results indicate that active GA biosynthesis is required for auxin to trigger fruit initiation and growth.

We also tested whether the expression of SIDELLA- and SIARF7/SIIAA9-target genes in *gib1* is responsive to GA and auxin treatment. As expected, both feedback- and growth-related downstream target genes in *gib1* responded to GA treatment in a manner similar to those in WT (Figure 7H). On the other hand, upon 2,4-D treatment, only the feedback-regulated genes were still responsive in *gib1*, while the expression of growth-related downstream genes was unaltered (Figure 7I). Therefore, under GA-deficiency conditions, auxin can still induce the expression of GA biosynthesis genes. However, in the absence of bioactive GA production, growth-related downstream target genes of SIDELLA/SIARF7/SIIAA9 are not responsive to auxin, as their expression is likely blocked by high levels of SIDELLA. To verify this idea, we tested whether SIDELLA protein levels are affected by auxin in the WT *GIB1* and *gib1* mutant backgrounds.

We used FLAG-DELLA transgenic lines, *FLAG-DELLA pro* and *FLAG-DELLA pro gib1*, for this analysis because FLAG-DELLA could readily be detected with an anti-FLAG antibody. As expected, FLAG-DELLA protein levels were much higher in *pro gib1* mutant than in *pro* due to the lack of bioactive GA in *gib1* (Figure 7J). For both *FLAG-DELLA* lines, emasculated –1 DAA ovaries were treated with 2,4-D or GA, harvested at different time points, and FLAG-DELLA levels analyzed by immunoblot analysis. Figure 7K shows that under mock treatment, DELLA protein levels in the *FLAG-DELLA pro* line increased significantly at 12 h after emasculation and remained high at 24 h. There were no significant changes in the DELLA transcript levels at these time points (Supplemental Figure 7), indicating that the increased DELLA protein accumulation at 12-24 h is due to increased DELLA stability caused by the lack of GA production after emasculation. Under 2,4-D treatment, DELLA protein levels greatly decreased 12 h after emasculation and remained low at 24 h (Figure 7K), although its transcript levels remain unaltered (Supplemental Figure 7). In the *FLAG-DELLA pro gib1* line, FLAG-DELLA protein levels did not change after emasculation in the mock controls (Figure 7L), likely because FLAG-
DELLA protein already accumulated to high levels in the *gib1* background due to GA deficiency. Importantly, auxin treatment did not reduce the levels of FLAG-DELLA accumulation in the *gib1* background (Figure 7L). GA treatment, which was used as a control, caused rapid degradation of FLAG-DELLA in both the *procera* and *pro gib1* backgrounds (Figures 7K and 7L). These results strongly suggest that auxin-induced tomato fruit initiation requires active GA biosynthesis and signaling to reduce the levels of the major repressor SIDELLA.

**DISCUSSION**

Our study revealed that SIDELLA and SIARF7/SIIAA9 mediate crosstalk between GA and auxin signaling pathways to regulate fruit initiation in tomato (Figure 8). SIDELLA induces the expression of GA biosynthesis genes (*GA20ox1* and *GA3ox1*) and *GH3.2* (encoding an auxin conjugating enzyme), which are known to be feedback-regulated genes by GA and auxin, respectively (Figure 8A). Co-expression of SIARF7 and SIIAA9, however, represses the expression of these feedback-regulated genes. Because SIARF7 and SIIAA9 directly interact, it is likely that the SIARF7/SIIAA9 complex functions as a transcriptional repressor to inhibit the transcription of these feedback-regulated genes (Figure 8A-8B). Our data also indicate that SIDELLA directly interacts with SIARF7 and other activator SIARFs and that the co-expression of SIDELLA and SIARF7/SIIAA9 antagonistically regulates the feedback-regulated genes involved in GA biosynthesis and auxin deactivation (Figure 8A). ChIP-qPCR analysis indicated that both SIDELLA and SIARF7 associate with chromatin containing the promoters of these feedback-regulated genes. Reduced SIDELLA levels by GA treatment led to slightly increased SIARF7 binding to these feedback-regulated promoters. Therefore, the SIDELLA-SIARF7 interaction may sequester SIARF7 away from these target gene promoters. We also found that SIDELLA binds to the middle region (MR) of SIARF7 instead of its DNA binding domain, suggesting that the SIDELLA-SIARF7 interaction may alter the conformation of SIARF7. SIDELLA may also recruit unidentified DNA-binding transcription factors to activate the transcription of these genes (Figure 8B); AtDELLAs activate the transcription of GA feedback-regulated genes through direct interactions with the zinc-finger transcription factors, INDETERMINATE DOMAIN family proteins (Fukazawa et al., 2014; Yoshida et al., 2014). A previous study (de Jong et al., 2011) also suggested that SIARF7 affects GA responses, as the morphology of parthenocarpic *SIARF7 RNAi* fruits was similar to that of GA-induced...
parthenocarpic fruits, and \textit{GA20ox1} mRNA levels were higher in \textit{SlARF7 RNAi} lines than in WT at anthesis. However, at 12 DAA, bioactive GA levels in the pericarps of parthenocarpic fruits of the \textit{SlARF7 RNAi} lines were lower than those of WT fruits (de Jong et al., 2011). At this later stage, the high levels of GAs in WT pericarp could be due to the transport of GAs from developing seeds.

In contrast to the antagonistic effects of \textit{SIDElla} vs. \textit{SlARF7/SlIAA9} on feedback-regulated genes, these proteins inhibit the expression of downstream growth-related genes (e.g. \textit{EXP5}) and induce the expression of ethylene biosynthesis gene \textit{ACO4} (Figure 8A), as shown by gene expression (Figures 6E-6F) and ChIP-qPCR (Figure 5G) analyses. Previous studies have indicated that the activator ARFs function as transcriptional activators when they form ARF-ARF dimers (Ulmasov et al., 1999; Hardtke et al., 2004). We propose that before pollination, \textit{SIDElla} and \textit{SlIAA9} inhibit the expression of growth-related genes during fruit set by binding to \textit{SlARF7} (and other activator \textit{SlARFs}) (Figure 8C). Interestingly, \textit{SlARF7} interacted with \textit{SIDElla} and \textit{SlIAA9} through distinct regions, and binding of \textit{SIDElla} or \textit{SlIAA9} did not affect the homo-dimerization of \textit{SlARF7} or its hetero-dimerization with other activator \textit{SlARFs}. These results suggest that the binding of \textit{SIDElla} and \textit{SlIAA9} to activator \textit{SlARFs} may directly inhibit their activity without interfering with their dimerization. Furthermore, our ChIP-qPCR data suggest that \textit{SIDElla} may enhance the binding of \textit{SlARF7} to downstream target promoters, whereas the association of \textit{SIDElla} with these targets is not affected by reduced levels of \textit{SlARFs} in the \textit{SlARF7 RNAi} line. One possibility is that \textit{SIDElla} also binds to other unidentified transcription factor(s), which stabilizes the binding of \textit{SlARF7} to target promoters but inhibits its transactivation activity (Figure 8C). Upon pollination, \textit{SIDElla} and \textit{SlIAA9} are degraded under elevated GA and auxin levels. This allows \textit{SlARF7} and other activator \textit{SlARFs} to activate the transcription of the growth-related genes. Using the GA-deficient \textit{gib1} mutant, we showed that auxin-induced tomato fruit initiation is GA-dependent. In the \textit{gib1} mutant background, elevated auxin signaling (via auxin treatment or \textit{e} mutation) did not remove the high levels of \textit{SIDElla} protein. Based on this observation, one may consider that auxin functions upstream of the GA pathway. However, the additive effect of \textit{e (iaa9)} and \textit{pro (della)} indicates that both auxin and GA signals are necessary to remove both the auxin signaling repressor (IAA9) and GA signaling repressor (DELLA). Our biochemical and genetic studies provide support for the molecular interaction between GA and auxin pathways during fruit set. Our results help
explain why both auxin and GA play important roles in fruit initiation and subsequent growth.

Upon fertilization, auxin produced in the tomato ovule induces increased GA biosynthesis, which in turn triggers DELLA degradation. At the same time, auxin induces the degradation of SlIAA9 (and possibly other SIIAAs), which is mediated by auxin receptors. Removing both key repressors (SIIAA and SIDELLA) releases SIARFs, allowing them to activate downstream genes that are important for fruit initiation and fruit growth.

The model presented in Figure 8 is supported by the observation that procera (della), SlARF7 RNAi, and entire (iaa9) have additive effects in promoting parthenocarpic fruit development, as shown by the phenotypes of the single and double mutants. In addition to SlARF7, it is likely that other activator SIARFs also play a role in controlling fruit set. We found that all activator SIARFs were expressed around anthesis, and they interacted with SIDELLA in Y2H assays. A recent study showed that amiRNA-induced downregulation of SlARF5 expression resulted in parthenocarpy (Liu et al., 2018). We also showed that besides SlARF7, SlARF5 and SlARF8B were downregulated in the SlARF7 RNAi line. Moreover, the CR-slrf7 slar5/+ double mutant, but not its corresponding single mutants, produced parthenocarpic fruits, although at a lower frequency compared to the ARF7 RNAi line (Figures 2F and 6D). In addition, the average parthenocarpic fruit size of this double mutant was much smaller than that of the ARF7 RNAi line (Figure 2G). However, the CR-slrf7 slar5/+ double mutant and the ARF7 RNAi line are in two different backgrounds (cultivar M82 vs. MM), which may also contribute to the differences in their phenotypes. It is possible that SlARF8B also plays a role in fruit set, as this gene is expressed at quite high levels around anthesis and its expression is downregulated in the SlARF7 RNAi line. Taken together, our results support the idea that SlARF5, SlARF7 and additional SIARFs play overlapping roles in the regulation of fruit set. Based on the results of these genetic analyses, it is intriguing that these activator SIARFs appear to inhibit auxin-induced parthenocarpy. Considering that SIIAA9 represses auxin signaling by binding to activator ARFs, mutations in SlARF7 and SlARF5 may allow other activator SIARFs to induce downstream target genes more effectively if they have lower affinity to SIIAA9 than SlARF7 and SlARF5.

Consistent with this idea, we found that SlARF8A and SlARF8B displayed weaker interactions with SIIAA9 than SlARF5 and SlARF7 in a Y2H assay (Figure 2A). As discussed above, our model also suggests that SlARF7 and other activator SIARFs promote fruit set when SIIAA9 and SIDELLA are degraded in response to elevated auxin and GA levels upon fertilization. The
generation and characterization of single and multiple knockdown and knockout lines for additional activator SIARFs should help elucidate the specific and complex roles of individual SIARFs in fruit initiation.

Using the tomato system, we demonstrated that direct crosstalk between GA and auxin signaling pathways plays a role in controlling fruit initiation. Although recent studies in Arabidopsis reported an interaction between DELLA and ARF in Y2H and co-IP assays (Oh et al., 2014), the physiological effect of the DELLA-ARF interaction in planta is difficult to examine in Arabidopsis by genetic analysis, mainly due to the functional redundancy among the five AtDELLAs and the large numbers of AtARFs and AtIAAs. Importantly, the tomato genome contains only a single SIDELLA (PROCERA) gene. Although tomato contains many IAA genes, the single entire (iaa9) mutant displays a clear parthenocarpic phenotype. These unique features of the tomato system allowed us to elucidate the genetic interactions among SIDELLA, SIIAA9 and the activator SIARFs during fruit initiation.

METHODS

Plant Materials, Growth Conditions and Statistical Analysis

Tomato (Solanum lycopersicum) cultivars Ailsa Craig (AC) and Moneymaker (MM), and the entire mutant [in the AC background, (Rick and Butler, 1956)] were obtained from the Tomato Genetics Resource Center at UC Davis. The procera (5x bc AC, Smith and Ritchie, 1983) and procera gib1 (Van Tuinen et al., 1999) mutants were gifts from Dr. Maarten Koornneef (Max Planck Institute for Plant Breeding Research, Germany), and the ARF7 RNAi line #4 (in the MM background) was generated by Dr. Wim Vriezen (de Jong et al., 2009). The AC/MM hybrid and double mutants ARF7 RNAi procera, entire procera, entire ARF7 RNAi, ARF7 RNAi gib1 and entire gib1 were generated by genetic crosses. The slarf5-1 (slmp-1, generated by EMS mutagenesis) and CR-slarf7 mutants are in the M82 cultivar background. The FLAG-DELLA procera gib1 line was produced by crossing the FLAG-DELLA procera transgenic line with procera gib1. Genotyping primers for the procera, entire, gib1, CR-arf7, arf5-1 mutants are listed in Supplemental Data Set 1.

Tomato plants were grown in the greenhouse with 16h/8h day/night light cycle. During the 16h photoperiod, supplemental lights (Phillips 1000w Hortilux Super HPS) were turned on when internal light intensity was below 900 µmol/m²/sec for 30 min. Lights were turned off
when internal light intensity exceeded 900 µmol/m²/sec for 30 min. Temperatures in the greenhouse were maintained at 21.1-26.7 °C/18.3-23.9 °C (day/night), and humidity was maintained between 37% and 72% RH. Flowers for emasculation and/or hormonal treatments were from the third to seventh flower trusses on the primary stem. For hormonal treatments, 10 µl of 300 µM GA₃, 100 µM 2,4-D, or mock solvent (5% MeOH, 0.1% Tween-20) was applied to emasculated –1 DAA ovaries.

All statistical analyses were performed using Student’s t-test.

Plasmid Construction

Primers for plasmid construction are listed in Supplemental Data Set 1. PCR-generated DNA constructs were sequenced to make sure no mutation was introduced. Detailed information on plasmid construction is described in the Supplemental Data Set 2.

Yeast Two-Hybrid and Three-Hybrid Assays

The ProQuest Two-Hybrid system (Invitrogen) and yeast strain pJ69-4A were used for the Y2H and Y3H assays. Yeast transformation and 3-Amino-1,2,4-triazole (3-AT) tests were carried out as described previously (Dill et al., 2004) with slight modifications: 3-AT concentrations in the plates were 0, 5, 10, 25, 50, 75 and 100 mM in most cases, although concentrations of 150, 200, 250 and 300 mM 3-AT were used when the interactions were strong. Immunoblot analyses were performed using anti-HA (1:2000, Covance #MMS-101P), anti-FLAG (1:5000, Sigma A8592) or anti-cMyc (1:2000, Covance #MMS-150P) primary antibodies to show that the HA-DNA-BD, 3xFLAG-AD fusions in the Y2H assays and Myc-fusions in the Y3H assays produced the expected proteins in yeast.

Plant Transformation

Transformation of the ProSLDELLA:FLAG-SIDELLA construct into the procera mutant was performed by NCSU Plant Transformation Laboratory (Raleigh, NC) with Agrobacterium tumefaciens strain GV3101 pMP90. T0 lines containing a single insertion were identified by their T1 seeds having a 3:1 ratio of kanamycin-resistant versus kanamycin-sensitive segregation
patterns. All eight independent homozygous lines identified in the T1 generation rescued procera defects; line #C1-18-6 was chosen for further study.

Transient expression (for co-IP and the dual luciferase assays) in wild tobacco (Nicotiana benthamiana) leaves was performed as described (Zentella et al., 2016). Transient expression in tomato fruits (for ChIP) was performed based on a previous report (Orzaez et al., 2006), with slight modifications. Agrobacterium strain 1D1249 (a gift from Dr. Gregory Martin, Cornell University) containing the pEG203-ARF7 plasmid was used for this test. Incubation of 1D1249 cells in induction solution was performed as described for N. benthamiana infiltration (Zhang et al., 2011). The bacterial infiltration solution was injected into 2-3 cm diameter WT fruits (2-3 weeks after anthesis) using a syringe with a 20-gauge needle; the needle was inserted into the stigma end of the fruit. When the needle was inserted half-way into the fruit, bacterial solution in the syringe was injected into the fruit until the solution emerged from the tips of sepals. Five days after infiltration, the jelly-like tissue in the fruit locules was harvested for ChIP-qPCR.

For the transient Myc-ARF7 expression +/-GA test, WT plants were drenched with 10 µM paclobutrazol 3 times a week for 2 weeks to increase DELLA levels in the fruit. One day before harvest (i.e. four days after infiltration), 300 µM GA₃ or mock solution was injected into Myc-ARF7 infiltrated fruits. Locular tissues were harvested on day 5 as described above. For transient Myc-DELLA ChIP, 1D1249 Agrobacteria cells containing pEG203-DELLA plasmid were injected into WT or ARF7 RNAi fruits.

Quantitative RT-PCR (qPCR)

Total RNA was isolated using a Quick-RNA MiniPrep kit (Zymo Research). First-strand cDNA was synthesized using a Transcriptor First Strand cDNA Synthesis kit (Roche Applied Science). For qPCR, FastStart Essential DNA Green Master mix (Roche Applied Science) was used on a Mastercycler ep realplex Instrument (Eppendorf). The PCR program consisted of an initial denaturation at 95°C for 10 min, then 45 cycles of amplification at 95°C for 10 sec, 60°C for 10 sec and 72°C for 15 sec, followed by melting curve step at 95°C for 10 sec, 65°C 60 sec, and increases temperature @ 0.1°C/sec to 95°C for 1 sec. Three biological replicates from independent pools of tissues (2 technical repeats each) were included for each experiment. Primers for qPCR are listed in Supplemental Data Set 1.
For absolute qPCR analysis, the qPCR standard curves of \textit{SIUBQ7} and \textit{SlARF} genes were generated as described before (Tyler et al., 2004). For \textit{SIUBQ7}, a 200-bp PCR product of \textit{SIUBQ7} cDNA amplified by primers \textit{SIUBQ7}-1 and \textit{SIUBQ7}-2 from AC cDNAs was first inserted to pCR8/GW. Then, linearized pCR8-SIUBQ7 DNA was used as template for determining cDNA copy vs. cycle number. For \textit{SlARFs}, the DNA templates were linearized plasmids containing pCR8-\textit{SIARF} cDNAs (except for \textit{SlARF7}, which was in pENTR1A). Based on these standard curves, the cDNA copy numbers of \textit{SlARF} genes and \textit{SIUBQ7} were determined according to their respective cycle numbers.

\textbf{Co-immunoprecipitation (co-IP) and Dual Luciferase Assay}

Co-IP assays using proteins transiently expressed in \textit{N. benthamiana} by agro-infiltration were performed as described previously (Zentella et al., 2016). The dual luciferase assays was also performed via transient expression in \textit{N. benthamiana}. First reporter plasmids (\textit{tomato gene promoter:fLUC} and \textit{Pro}\textsubscript{35S}:\textit{rLUC}) and effector constructs (\textit{Pro}\textsubscript{35S}:\textit{ARF7}, \textit{DELLA} or \textit{IAA9} in pEarleyGate vector) were separately transformed into agrobacterium strain GV3101. The \textit{fLUC} and \textit{rLUC}-containing strains were co-infiltrated into leaves, with various agro combinations of \textit{ARF7}, \textit{DELLA}, \textit{IAA9} constructs. Two days after infiltration, \textit{N. benthamiana} leaves were harvested for protein extraction, and luciferase activity was measured using the dual-luciferase reporter assay system (Promega). Relative promoter activity was calculated as the ratio of \textit{fLUC} to \textit{rLUC} activity for each sample. Nine biological repeats (three independent pools of tissue were assayed at one time, and repeated at 3 different times) were conducted for each effector combination.

\textbf{ChIP-qPCR}

The ChIP-qPCR experiment was performed as described (Zentella et al., 2007), with slight modifications. For FLAG-SIDELLA ChIP, –1 DAA ovaries of the FLAG-SIDELLA \textit{procera} transgenic tomato line were used. For transient SIARF7 ChIP, Myc-SIARF was transiently expressed in 2-3 cm diameter WT fruits (2-3 weeks after anthesis) and the locule tissues were harvested and crosslinked for ChIP. Non-transgenic tissues (\textit{procera} for SIDELLA ChIP, and WT + 1D1249 for SIARF7 ChIP) were included as controls. There were two modifications for the transient SIARF7 +/- GA ChIP. Firstly, the WT plants were watered with
10 µM PAC for two weeks. Secondly, GA solution was injected into fruit one day before harvest. Non-transgenic tissues (WT/PAC + 1D1249 -GA) were included as a control. For transient SIDELLA ChIP, Myc-SIDELLA was expressed in WT or SLARF7 RNAi fruit. 1D1249-infiltrated WT fruit was used as a control.

After crosslinking the tissues, chromatin was extracted as described in Zentella et al. (2007), and DNA was eluted from antibody-conjugated beads with modified elution buffer (20 mM Tris-HCl, pH 7.5, 5 mM EDTA, 50 mM NaCl, 1 % SDS, 50 µg/ml proteinase K). qRT-PCR was then performed using primers specific for different regions of the target promoters. The SlUBQ7 gene (Solyc10g005560) was used to normalize the qPCR results in each ChIP sample. Primer sequences are listed in Supplemental Data Set 1.

**Histology**

After emasculation of flowers, ovaries were collected from gib1, ARF7 RNAi gib1 or entire gib1 plants. Tissue fixation was performed as described (Hu et al., 2008), followed by tissue embedding in Technovit 7100 (Heraeus Kulzer GmbH) according to the manufacturer’s protocol. Three µm sections produced with a microtome (model 820, Spencer Lens Co., NY) were stained with 0.1% toluidine blue in 1% borax and examined by bright-field microscopy as described (Hu et al., 2008).

**In Vitro IAA-Aspartic Acid Conjugation Assay**

Recombinant GST, GST-SlGH3.2 and GST-AtGH3.6 were expressed in E. coli BL21-CodonPlus (DE3)-RIL (Agilent Technologies). Induction and purification of recombinant proteins were performed as described in Zentella et al. (2016). The *in vitro* conjugation of IAA-Aspartic acid and subsequent detection procedures basically followed a previous report (Staswick et al., 2005), with slight modifications. In particular, the conjugation reaction was carried out at room temperature overnight in 50 µl solution containing 500 ng GST fusion proteins or GST. 6 µl reactions were analyzed by thin layer chromatography (TLC), and staining of IAA or IAA-Asp was done with Salkowski reagent.

**Accession Numbers**
Sequence information for genes included in this article can be found in the Sol Genomics Network and GenBank (for tomato genes) and TAIR network (https://www.arabidopsis.org/; for Arabidopsis genes) under the following accession numbers: **SIDELLA** (i.e. **PROCERA**, Solyc11g011260), **SLARF7** (Solyc07g042260), **SIIAA9** (i.e. **ENTIRE**, Solyc04g076850), **SLARF5** (Solyc04g084210), **SLARF8A** (Solyc03g031970), **SLARF8B** (Solyc02g037530), **SLARF19A** (Solyc07g016180; NM_001247811), **SLARF19B** (Solyc05g047460; XM_010322983), **SLARF1** (Solyc01g103050), **SLARF2A** (Solyc03g118290), **SLARF2B** (Solyc12g042070), **SLARF3** (Solyc02g077560), **SLARF4** (Solyc11g069190), **SLARF9A** (Solyc08g082630), **SLARF10A** (Solyc11g069500), **SLARF16A** (Solyc09g007810), **SLARF24** (Solyc05g056040), **SIGA20ox1** (Solyc03g006880), **SIGA3ox1** (Solyc06g066820), **SIGH3.2** (Solyc01g107390), **SLAG1** (Solyc02g071730), **SITM29** (Solyc02g089200), **JOINTLESS** (Solyc11g010570), **SIMADS6** (Solyc01g093960), **SIFUL1** (Solyc06g069430), **SIMADS29** (Solyc11g005120), **SICYCB1;2** (Solyc10g080950), **SIEXP5** (Solyc02g088100), **SIEXP12** (Solyc05g007830), **SLXTH9** (Solyc12g011030), **SIXTH15** (Solyc03g031800), **SLABI2** (Solyc07g040990), **SIACO4** (Solyc02g081190), **SIERF1B** (Solyc09g066360), **SIcps** (i.e. **GIB1**, Solyc06g084240), **SLUBQ7** (Solyc10g005560), **AtARF5** (At1g19850), **AtCPS** (At4g02780), **AtGH3.6** (At5g54510).

**Supplemental Data**

**Supplemental Figure 1.** Activator SLARF hetero-dimerization is not affected by SLDELLA or SIIAA9 in a Y3H assay.

**Supplemental Figure 2.** Standard curves for absolute quantification of transcript levels by qPCR.

**Supplemental Figure 3.** GH3.2 functions as an amino acid-IAA conjugating enzyme *in vitro.*

**Supplemental Figure 4.** *PROCIDELLA:*FLAG-DELLA is functional in tomato.

**Supplemental Figure 5.** SLDELLA protein in fruit locule tissue accumulated to higher levels in response to PAC treatment and was completely degraded 24 hr after GA3 application.

**Supplemental Figure 6.** Response of putative GA/auxin target genes to GA or 2,4-D treatment in ovaries.

**Supplemental Figure 7.** SLDELLA transcript levels in emasculated -1 DAA ovaries compared to mock and hormone treatments.

**Supplemental Data Set 1.** List of primers and their uses.
Supplemental Data Set 2. Constructs.

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We thank Maarten Koornneef for providing procera and procera gib1 mutants, Wim Vriezen and Ivo Rieu for the SLARF7 RNAi line, Miltos Tsiantis and Klaus Theres for providing pGPTV-Kan-PROCERA, Zachary B. Lippman for identifying the slmp-I mutation, and Yossi Capua and Yuval Eshed for providing the SLARF7 construct. We also thank Gregory Martin for help with tomato transient expression, Neelima Sinha and Siobhan Brady for sharing protocols and tomato sequence information, George Allen and Sergei Krasnyanski at the Plant Transformation Laboratory at NC State University for tomato transformation, Rodolfo Zentella, Ning Sui and Jonathan Dayan for generating the modified Y2H vectors, and Jiayu Chen for technical assistance. This work was supported by grants to T.S. (US Department of Agriculture 2010-65116-20460, 2014-67013-21548 and 2018-67013-27395, National Institutes of Health R01 GM100051, and National Science Foundation MCB-0923723).

AUTHOR CONTRIBUTIONS

J.H. and T.S. designed the research; J.H. performed most experiments; N.O. identified the slarf5-I mutant; A.I. and N.O. generated the CR-slarf7 single mutant and the CR-slarf7 slarf5-I/+ double mutant; J.H and T.S. analyzed and wrote the manuscript.

REFERENCES


Figure 1. SIARF7 interacted with SIDELLA and SIIAA9 through distinct domains.

(A) Y2H assay showing that SIDGELLA directly interacted with SIARF7.

(B) Co-IP of transiently expressed HA-SIDGELLA with Myc-SIARF7 or Myc-GUS in tobacco. Protein extracts were immunoprecipitated with anti-Myc antibody-conjugated agarose beads. Immunoblot analysis was performed using anti-Myc or anti-HA antibodies. Asterisk marks the full-length Myc-ARF7, and lower bands are truncated Myc-ARF7.

(C) Y2H assay showing that SIIAA9 interacted with SIARF7, but not with SIDGELLA.

(D) SIDGELLA and SIIAA9 did not compete for binding with ARF7 in Y3H assay.

(E) Diagram of the full-length and various truncated SIARF7 used in (F). DD: dimerization domain; DBD: DNA-binding domain; AD: activation domain; PB1: Aux/IAA binding domain.

(F) SIDGELLA and SIIAA9 bound to different regions of SIARF7 in Y2H assay.

(G) Y3H assay showed that SIDGELLA or SIIAA9 did not interfere with ARF7 dimerization.

In (A, C, F), + His: synthetic medium minus tryptophan, leucine. – His: synthetic medium minus tryptophan, leucine and histidine, supplemented with 10, 50, 75 or 100 mM 3-AT, as labeled. In (D, G), +His and –His media are the same as indicated above, except that uracil was not included in these media. In (A, D, F), SIDGELLA-CT3 (amino acid residues 166-588) was used as the bait.
Figure 2. Several activator SIARFs likely function in fruit set.
(A) Activator SIARFs interacted with SIDELLA and SIIAA9 in Y2H assay. DELLA-CT3 (amino acid residues 166-588) was used as the bait. +His: synthetic medium minus tryptophan, leucine. –His: synthetic medium minus tryptophan, leucine and histidine.
(B) Temporal expression patterns of activator SIARFs in ovaries around anthesis. Absolute transcript levels of activator SIARFs in WT (MM) ovaries from -2 to +3 DAA.
(C) Absolute transcript levels of activator SIARFs in 0 DAA ovaries of WT and ARF7 RNAi line. The mRNA levels in (B) and (C) were calculated using standard curves (Supplemental Fig. 2), and are shown as the copies of SIARF cDNA per 2x10² and 10² copies of SIUBQ7, respectively.
(D) Expression of repressor SIARFs was not down-regulated in the ARF7 RNAi line. Relative repressor SIARF mRNA levels in WT or ARF7 RNAi 0 DAA ovaries were determined by RT-qPCR analysis. SIUBQ7 was used to normalize different samples. The level of each repressor SIARF in WT was set to 1.
In (C) and (D), Means ± SE of 3 biological replicas from independent pools of tissues (2 technical repeats each) are shown. *p < 0.05; **p < 0.01.
(E-G) The CR-slarf7 slarf5/+ double mutant produced parthenocarpic fruits. In (E), bar = 1cm. In (F), parthenocarpy efficiency as calculated by % of parthenocarpic fruits developed from emasculated flowers. In (G), average sizes of fruits from pollinated and emasculated WT and mutant flowers are shown. In (F-G), means ± SE from three biological repeats (sampled at different times). n=21-85 for each line are shown. Different letters above bars represent significant differences among emasculated lines, p < 0.01.
Figure 3. **SIDELLA and SIARF7/SIIAA9 oppositely regulated GA- and auxin-feedback genes.**

(A) WT flower morphology around anthesis is similar to previously described (Brukhin et al., 2003). DAA: day after anthesis. Flowers were harvested from WT plants at different developmental stages without further manipulation. Bar: 1cm.

(B-E) Relative mRNA levels in –1 DAA ovaries as determined by RT-qPCR analysis. A housekeeping gene, *SlUBQ7*, was used to normalize different samples. Means ± SE of 3 biological replicas from independent pools of tissues (2 technical repeats each) are shown. The level in mock treatment (B-C) or WT (D-E) was set to 1. * p < 0.05; ** p < 0.01. In (B) and (C), –1 DAA ovaries of emasculated flowers were treated with hormone or mock treated. (B) and (D) GA biosynthetic genes (*GA20ox1, GA3ox1*) and amino acid-auxin conjugating gene (*GH3.2*) were repressed after 6 hr GA3 treatment or in the *procera* mutant (comparing to that in WT). (C) and (E) *GA20ox1, GA3ox1* and *GH3.2* were all induced after 6 hr 2,4-D treatment (C), and in elevated auxin-signaling mutants *ARF7 RNAi* and *entire* (*iaa9*) (E).

(F-H) Dual luciferase assay in the tobacco transient expression system showed that SIDELLA and SIARF7/SIIAA9 antagonistically regulate expression of *GA20ox1* (F), *GA3ox1* (G), and *GH3.2* (H) promoters. Different letters above graph bars represent significant differences (p < 0.01). The reporter constructs contain 2.5 kb *GA20ox1* promoter (in F), 2.4 kb *GA3ox1* promoter (in G) and 2.5 kb *GH3.2* promoter (in H) that are fused to the *fLUC* coding sequence. In (F-H), effector constructs used in each assay are as labeled, and the empty effector construct was included as a negative control.
Figure 4. Direct association of SIDELLA and SIARF7 with promoters of GA/auxin feedback genes by ChIP-qPCR.

(A) Diagrams of SlGA20ox1, SlGA3ox1 and SlGH3.2 promoters. qPCR amplicons for ChIP-qPCR are depicted as short lines under the promoters. ↓ points to the position of canonical AuxRE (TGTC) elements in these promoters. ⇧ indicates translational start sites.

(B) SIDELLA bound to promoter regions of GA20ox1, GA3ox1 and GH3.2 genes in vivo. Chromatin isolated from crosslinked -1 DAA ovaries of the PSlDELLA:FLAG-SlDELLA transgenic tomato line was immunoprecipitated using anti-FLAG antibodies and followed by qPCR.

(C) Myc-SlARF7 bound to GA and auxin feedback-regulated genes by ChIP-qPCR. 35S:Myc-SlARF7 was transiently expressed in tomato fruits by agro-infiltration, and ChIP was performed using anti-Myc antibodies and followed by qPCR.

(D) ChIP-qPCR analysis showed GA treatment enhanced SlARF7 binding to GA20ox1 and GH3.2 promoters, presumably because of reduction in DELLA protein levels. Myc-ARF7 was transiently expressed as in (C).

(E) Myc-SIDELLA protein that transiently expressed in WT or SlARF7 RNAi line showed equal binding to the promoters of feedback-regulated genes by ChIP-qPCR analysis.

In (B-E), the relative enrichment was calculated by normalizing against ChIP-qPCR of non-transgenic control samples using SlUBQ7. The normalized values of fold enrichment are the average ± SE of three biological replicas from independent pools of tissues. * p < 0.05; ** p < 0.01.
Figure 5. SIDE LL A and SIARF7/ SIIAA9 have similar effects on their direct downstream target genes, EXP5 and ACO4.

(A-B) Relative mRNA levels in –1 DAA ovaries as determined by RT-qPCR analysis. A housekeeping gene, SlUBQ7, was used to normalize different samples. Means ± SE of 3 biological replicas (2 technical repeats each) are shown. The level in mock treatment (A) or WT (B) was set to 1. * $p < 0.05$, ** $p < 0.01$. EXP5 was induced after 6 hr GA$_3$ or 2,4-D treatment (A), and in the procera, ARF7 RNAi and entire (iaa9) mutants (B, comparing to that in WT). In contrast, ACO4 was repressed by GA or 2,4-D treatment (A) and in procera, ARF7 RNAi and entire (iaa9) mutants (B).

(C) Diagrams of SlEXP5, SlACO4 promoters. qPCR amplicons for ChIP-qPCR are labeled as short lines under each diagram. ! points to the position of canonical AuxRE (TGTC) elements in these promoters. r→ indicates translational start sites.

(D) SIDE LL A bound to promoter regions of EXP5 and ACO4 genes in vivo by ChIP-qPCR analysis. Chromatin isolated from crosslinked –1 DAA ovaries of the P$_{SIDE LL A}$-FLAG-SIDE LL A tomato line was immunoprecipitated using anti-FLAG antibodies and followed by qPCR.

(E) Binding of transiently expressed Myc-SIARF7 to EXP5, ACO4 genes by ChIP-qPCR. 35S:Myc-SIARF7 was transiently expressed in tomato fruits by agro-infiltration, and ChIP was performed using anti-Myc antibodies and followed by qPCR.

(F) Myc-SIDE LL A protein that transiently expressed in WT or SIARF7 RNAi line showed equal binding to the promoters of downstream target genes by ChIP-qPCR analysis.

(G) ChIP-qPCR analysis showed GA treatment reduced SIARF7 binding to EXP5 and ACO4 promoters, presumably because of reduction in DELLA protein levels. Myc-ARF7 was transiently expressed as in (E). In (D-G), the relative enrichment was calculated by normalizing against ChIP-qPCR of non-transgenic control samples using SlUBQ7. The normalized values of fold enrichment are the average ± SE of three biological replicas from independent pools of tissues. * $p < 0.05$; ** $p < 0.01$. 

![Figure 5](image-url)
Figure 6. SIDELLA, SIARF7 and SIIAA9 additively repress fruit initiation and growth.
(A) and (B) Parthenocarpic growth in procera, entire, and ARF7 RNAi single and double mutants. WT cv. AC: Ailsa Craig. MM: Moneymaker. AC/MM: AC/MM hybrid. Pictures were taken four weeks after pollination (P) or emasculation (E). In (A), bar = 1 cm. In (B), bar = 0.5 cm. Pericarp (PC), locule tissue (LT), seed (S), placenta (PT) and columella (C).
(C) Average sizes of fruits from pollinated and emasculated WT and mutant flowers. Means ± SE (n=26-32 for pollinated fruits; 17-20 for parthenocarpic fruits). Different letters above bars represent significant differences among parthenocarpic fruits, p < 0.01.
(D) Parthenocarpy efficiency as calculated by % of parthenocarpic fruits developed from emasculated flowers. Means ± SE. n=18-28 for most lines, but n=41-50 for mutants with low parthenocarpy efficiency, including ARF7 RNAi, entire and e ARF7i. Different letters above bars represent significant differences, p < 0.05.
(E) and (F) Transcript levels of EXP5 and ACO4 in –1 DAA ovaries of single and double mutants. Means ± SE of 3 biological replicas (2 technical repeats each) are shown. Different letters above bars represent statistical differences (p < 0.01).
Figure 7. Fruit initiation by auxin requires active GA signaling

(A) Parthenocarpy was induced by exogenous GA and/or auxin application on WT -1 DAA ovary. Pictures were taken three weeks after pollination or emasculation + hormone treatment (bar: 1 cm).

(B-D) In the GA biosynthetic mutant gib1, parthenocarpy was induced by application of GA_3, but not by 2,4-D. -1 DAA ovaries of emasculated flowers were treated with mock solution or hormones as labeled. Three weeks after treatments, picture of ovaries/fruits was taken (B), average diameters of ovaries were measured (C), and cellular structures were analyzed by cross sectioning (D). In (B), bar: 1 cm. In (D), bar: 100 µm. In (C), means ± SE (n=16).

(E-G) ARF7i gib1 and e gib1 double mutants were unable to produce parthenocarpic fruits. Flowers were emasculated at -1 DAA. Five weeks after emasculation, picture was taken (E, bar: 5 mm), fruit diameters were measured (F). In (F), means ± SE (n=30), and different letters above bars represent significant differences (p < 0.01). Cellular structures were analyzed by cross sectioning (G, bar: 100 µm).

(H-I) In gib1, DELLA/ARF7/IAA9 downstream target genes (EXP5, ACO4) did not respond to 2,4-D treatment (I), while feedback-related genes still did. In contrast, all genes responded to GA_3 in the same manner as in WT (H). Values are means ± SE of 3 biological replicas (2 technical repeats each). ** p < 0.01.

(J) FLAG-DELLA protein accumulated to a higher level in pro gib1 than in the pro background.

(K) 2,4-D application on emasculated -1 DAA ovary of FLAG-DELLA pro line reduced SIDEELLA protein levels gradually over 24-hr period. DELLA levels consistently increased 12 hr after mock treatment, while GA application caused DELLA degradation in 1 hr.

(L) Both 2,4-D and mock treatments on emasculated -1 DAA ovary of FLAG-DELLA pro gib1 line did not affect DELLA levels over 24-hr period. In contrast, GA still caused degradation of DELLA as in pro.

In (J-L), FLAG-DELLA was detected by anti-FLAG antibody, and blots probed with α-tubulin were included to show equal loading.
Figure 8. Model of crosstalk between early GA and auxin signaling pathways during tomato fruit initiation.

(A) Schematic of GA and auxin pathways. The SlARF7/SlIAA9 complex functions as an auxin signaling repressor complex, and SlDELLA is a GA signaling repressor. They additively inhibit tomato fruit set and fruit development by repressing growth-related genes (e.g. EXP5) and activating ethylene biosynthesis gene (ACO4). In contrast, SlARF7/SlIAA9 and SlDELLA antagonistically regulate expression of feedback-regulated genes, including GA biosynthesis genes (GA20ox1 and GA3ox1) and auxin deactivation gene (GH3.2). Upon pollination, auxin and GA play key roles in fruit set. Auxin levels increase in the fertilized ovule, which in turn induces IAA9 degradation and GA biosynthesis. Elevated GA levels then trigger SlDELLA degradation. Removing both SlDELLA and SlIAA9 promotes fruit set and subsequent development.

(B) Molecular model for regulation of feedback-regulated genes by SlDELLA and SlARF7/SlIAA9. Feedback-regulated genes in GA and auxin metabolism are repressed by SlARF7/SlIAA9 complex, but are induced by SlDELLA. Binding of SlDELLA to SlARF7 may sequester SlARF7 from the target gene promoters. SlDELLA may also recruit unidentified transcription factors (?) to activate transcription of these genes.

(C) Molecular model for regulation of downstream fruit development-related genes by SlDELLA and SlARF7/SlIAA9. Fruit set and growth-related genes are repressed by inhibition of SlARF7 activity by SlDELLA and SlIAA9 via direct protein-protein interactions. SlDELLA may also bind to unidentified transcription factor (?), which stabilizes SlARF7 binding to target promoters but inhibits its transactivation activity. Elevated levels of auxin and GA in fertilized ovule can trigger degradation of SlIAA9 and SlDELLA, respectively. This then allows SlARF7 (and additional activator SlARFs) to activate transcription of downstream growth-related genes.
DELLA-ARF/IAA Interaction Mediates Crosstalk between Gibberellin and Auxin Signaling in Controlling Fruit Initiation in Solanum lycopersicum

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