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RESEARCH PAPER

Functional characterization of a gibberellin F-box protein, PslSLY1, during plum fruit development

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Abstract

Fruit development is orchestrated by a complex network of interactions between hormone signaling pathways. The phytohormone gibberellin (GA) is known to regulate a diverse range of developmental processes; however, the mechanisms of GA action in perennial fruit species are yet to be elucidated. In the current study, a GA signaling gene *PslSLY1*, encoding a putative F-box protein that belongs to the *SLY1* (SLEEPY1)/*GID2* (gibberellin-insensitive dwarf2) gene family, was isolated from Japanese plum (*Prunus salicina*). *PslSLY1* transcript abundance declined as fruit development progressed, along with potential negative feedback regulation of *PslSLY1* by GA. Subcellular localization and protein–protein interaction assays suggested that PslSLY1 functions as an active GA signaling component that interacts with the ASK1 (*Arabidopsis* SKP1) subunit of an SCF–ubiquitin ligase complex and with PslDELLA repressors, in a GA-independent manner. By using a domain omission strategy, we illustrated that the F-box and C-terminal domains of PslSLY1 are essential for its interactions with the downstream GA signaling components. *PslSLY1* overexpression in wild-type and *Arabidopsis sly1.10* mutant backgrounds resulted in a dramatic enhancement in overall plant growth, presumably due to triggered GA signaling. This includes germination characteristics, stem elongation, flower structure, and fertility. Overall, our findings shed new light on the GA strategy and signaling network in commercially important perennial crops.

Keywords: F-box protein, fruit development, functional biology, GA signaling, protein–protein interaction, *Prunus salicina*, subcellular localization.

Abbreviations: BiFC, bimolecular fluorescence complementation; DAB, days after bloom; EG, Early Golden; GA, gibberellin; WT, wild type; Y2H, yeast two-hybrid.

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Introduction

Fruiting is a distinct feature in flowering plants and represents an important research field because of its significance for human nutrition. Post-fertilization, fruits develop rapidly via a coordination of hormonal processes, leading to wide irreversible molecular, biochemical, and structural changes. The distinctive metabolic pathways that synchronize the progression of fruit development have been the subject of considerable research in a wide range of fruit species (Seymour *et al.*, 2013; Kumar *et al.*, 2014). The availability of naturally occurring and induced mutants in fruit crops has greatly assisted the elucidation of mechanisms underlying the progression in fruit development. Phytohormones and their dynamic interplay act as master regulators of fruit growth and development (Ozga and Reinecke, 2003; McAtee *et al.*, 2013). The phytohormone gibberellin (GA) is a major plant hormone that is involved in the coordination of a diverse range of functions (Richards *et al.*, 2001; Olszewski *et al.*, 2002; Thomas and Sun, 2004; Fleet and Sun, 2005). Levels and accumulation sites of GA and GA responsiveness are critical for flower induction and floral organ determination, as well as for fruit initiation, development, and ultimate fruit quality (Blázquez *et al.*, 1998; Goto and Pharis, 1999; Bernier and Perilleux, 2005; Mesejo *et al.*, 2013; El-Sharkawy *et al.*, 2014*a*; Acheampong *et al.*, 2015). Fertilization mutually triggers GA, auxin, and cytokinin pathways in ovules to initiate the flower-to-fruit transition program as well as the onset of the cell division and expansion processes associated with early embryo development (Vivian-Smith and Koltunow, 1999; Ozga and Reinecke, 2003; Davies, 2004; de Jong *et al.*, 2009; Mariotti *et al.*, 2011). Previous studies have demonstrated that GA levels increase commensurate with the progression of fruit development and growth (Bukovac and Nakagawa, 1967; Jackson, 1968; Yamaguchi and Takahashi, 1976; Looney and Pharis, 1986; Talon *et al.*, 1992; El-Sharkawy *et al.*, 2014*a*). On the other hand, GA-deficient mutant fruits present several developmental disorders, including malformed flowers, reduced fruit size, and delayed ripening (Walser *et al.*, 1981; Serrani *et al.*, 2007; de Jong *et al.*, 2009; El-Sharkawy *et al.*, 2012). While these studies suggested the important contribution of GA in fruit growth (Jackson, 1968; Crane, 1969; Bukvoac and Yuda, 1979; Gillaspy *et al.*, 1993), much of the fundamentals of the mechanism of GA action in these processes remain to be elucidated.

Plants maintain complex GA signaling networks to accommodate the diverse range of often simultaneous responses mediated by the hormone. The characterization of GA-defective mutants, displaying numerous traits, has helped researchers to recognize several crucial GA signaling pathways and outcomes (Magome *et al.*, 2004; Yasumura *et al.*, 2007; Yamaguchi, 2008; Harberd *et al.*, 2009; Olimpieri *et al.*, 2011). The most profound discovery has been the identification of the central GA signaling components in plants. The identification of the soluble GA receptor, GID1, the GA suppressor regulators, DELLA, and the GA activator F-box proteins, SLY1 and SNE (SNEEZY) in *Arabidopsis* or GID2 and SNE in rice, has improved our fundamental understanding of how GA mediates its activity (Peng *et al.*, 1997; Silverstone *et al.*, 1998; McGinnis *et al.*, 2003; Sasaki *et al.*, 2003; Strader *et al.*, 2004; Ueguchi-Tanaka *et al.*, 2005). At low GA levels, DELLA proteins halt the activity of the GA-responsive, DNA-binding basic helix–loop–helix (bHLH) transcription factors (de Lucas *et al.*, 2008; Feng *et al.*, 2008). GA perception by the GID1 receptor stimulates the interaction of GID1 with the DELLA proteins (Ueguchi-Tanaka *et al.*, 2005; Griffiths *et al.*, 2006), and the establishment of a stable co-receptor GA–GID1–DELLA complex results in conformational changes in DELLA proteins, facilitating SLY1/GID2 F-box and DELLA protein binding. Subsequent DELLA binding to the ubiquitin ligase complex $SCF^{SLY1/GID2}$ led to DELLA ubiquitination and degradation. The loss of DELLA promotes plant growth due to the release of GA-responsive genes from their restraint interactor (Griffiths *et al.*, 2006; Hirano *et al.*, 2010).

F-box proteins are subunits of the SCF E3 ubiquitin ligase complex and play diverse roles in plant development (Zheng *et al.*, 2002; Hare *et al.*, 2003; Zhang *et al.*, 2019). In GA signaling, the F-box domain binds to the Skp1 homolog, which conjugates the SLY1/GID2 proteins to Cullin, the core of the SCF complex (Dill *et al.*, 2004; Fu *et al.*, 2004). SLY1/GID2 relies on its C-terminal regulatory (GGF and LSL) domain to bind DELLA proteins via their C-terminal GRAS (VHIID and LHRII) domain (Dill *et al.*, 2004; Fu *et al.*, 2004; Hirano *et al.*, 2010; Ariizumi *et al.*, 2011). DELLA proteins are subsequently ubiquitinated by the E2/E3 ubiquitin ligase pathway via the $SCF^{SLY1/GID2}$ complex, and the ubiquitinated DELLA is then degraded via the 26S proteasome with the concomitant discharge of the ubiquitin moieties for reuse (Ariizumi *et al.*, 2011; Gao *et al.*, 2011; Sun, 2011). Although studies in *Arabidopsis* and rice have revealed a conserved GA signaling pathway across land plant lineages, the fact that each plant carries a distinct set of GA signaling paralogs adds extra complexity to GA response mechanisms. For instance, the GA signaling machinery in *Arabidopsis* consists of three GID1s, five DELLAs, and two SLY-like proteins (SLY1 and SNE). In contrast, the rice genome has only one representative of each of GID1 and DELLA, and two GID2-like proteins (GID2 and SNE) (Hauvermale *et al.*, 2012). Two GID1s, three DELLAs, and three SLY-like proteins (two SLY1 and one SNE) have been identified in grape (Acheampong *et al.*, 2015). Despite the dissimilarities, genetic and biochemical characterization has shown that all these paralogs are active and exhibit diverse overlapping and distinct roles in regulating different aspects of GA responses.

In the current study, a novel *SLY*-like gene, *PslSLY1*, encoding a putative F-box protein of the *SLY1/GID2* gene subfamily was isolated from Japanese plum. An analysis of

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transcript accumulation during fruit development suggested that *PslSLY1* is potentially subject to feedback regulation by GA. Assessing the localization and interaction properties of PslSLY1 and truncated versions of the protein revealed that PslSLY1 could influence GA responsiveness via the formation of an $SCF^{PslSLY1}$ ubiquitin ligase complex that presumably targets different DELLA repressors for degradation in a GA-independent manner. Overexpression of *PslSLY1* in *Arabidopsis* showed that the protein is able to re-establish GA responsiveness in the wild type (WT) and the GA-insensitive mutant, *sly1.10*. The presented data provide further evidence for the conserved role of GA in the organization and the progression of fruit development, and shed light on the complex regulatory processes involved in fruit growth.

Materials and methods

Plum tissues and treatments

Flowers and fruit at different developmental stages were collected from Japanese plum (*Prunus salicina* L.), cultivar Early Golden (EG), as described previously (El-Sharkawy *et al.*, 2007). To explore the influence of GA on *PslSLY1* mRNA accumulation, ripe fruits were picked before the onset of climacteric ethylene production, then sterilized, and subjected to treatment with gibberellin (GA_3, 100 μM) and the GA biosynthesis inhibitor paclobutrazol (PAC, 10 μM). Untreated fruits were used as controls. After the treatments, all fruit were stored at room temperature and samples were collected at 0, 3, 6, and 9 d post-treatment for analysis. A defined mix of tissues from three replicate fruits was sampled and frozen for later analysis at each time point.

Isolation and analysis of the PslSLY1 sequence

Based on common structural characteristics among previously characterized *SLY1/GID2*-like F-box genes, a pair of degenerate primers (#1 and 2, Supplementary Table S1 at *JXB* online) were designed to amplify a fragment of the plum gene ortholog. The putative *PsISLY1* amplicon was cloned, sequenced, and analyzed using BLAST (Altschul *et al.*, 1997). Elongation of the partial cDNA clone was carried out using the 3'- and 5'-RACE kit (Life Technologies, Grand Island, NY, USA). Full-length amplification of the *PslSLY1* cDNA sequence was carried out using Platinum Taq DNA Polymerase High Fidelity, following the instructions provided by the manufacturer (Life Technologies). Alignment of predicted proteins was performed using ClustalX software, and the Neighbor–Joining tree was generated with MEGA5 (Tamura *et al.*, 2011). Structural domains were annotated using the online software 'Smart' (Letunic *et al.*, 2009). The full-length genomic sequence (*gPslSLY1*) was isolated using the Universal Genome Walker Kit (Clontech, Palo Alto, CA, USA).

DNA and RNA extraction, and qPCR assays

Genomic DNA was extracted from young EG plum leaves according to the DNeasy Plant Mini Kit (Qiagen, Germantown, MD, USA). Total RNA extraction, DNase treatment, cDNA synthesis, and quantitative PCRs (qPCRs) were performed as described previously (El-Sharkawy *et al.*, 2012). Gene-specific primers were designed using Primer Express (v3.0, Applied Biosystems, Carlsbad, CA, USA) (primers #3–14, Supplementary Table S1). Three technical replicates from three biological replicates for each reaction were analyzed on an ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA). Transcript abundance was quantified using standard curves for both target and reference genes [*PslAct* (EF585293) and *AtAct* (NM_121018)], as described previously (El-Sharkawy *et al.*, 2012). The data were presented as an average of nine replicates (±SD).

Transient expression of PslSLY1–GFP fusion proteins in tobacco protoplasts

Truncated versions of PslSLY1 proteins were generated as described previously (El-Sharkawy *et al.*, 2014*b*). ORFs containing independent N-terminal ($PslSLY1_{\Delta N}$), F-box ($PslSLY1_{\Delta F}$), and C-terminal ($PslSLY1_{\Delta C}$) deletions of *PslSLY1* were generated by PCR using gene-specific primer pairs (primers #15–22, Supplementary Table S1). Full-length and truncated *PslSLY1* ORFs were fused in-frame with the green fluorescent protein (GFP) into the pGreenII vector using the *Bam*HI site and expressed under the control of the 35S promoter. Protoplasts used for transfection were obtained from suspension-cultured tobacco *BY-2* cells. Protoplasts were transfected with the constructs and analyzed for GFP fluorescence by confocal microscopy as described previously (El-Sharkawy *et al.*, 2009).

Yeast two-hybrid (Y2H) assay

Y2H assays were carried out using the Matchmaker Gold Yeast two-hybrid System (Clontech) as described previously (El-Sharkawy *et al.*, 2014*a*). Full-length and truncated *PslSLY1* ORFs were inserted into the *Bam*HI–*Pst*I site of the pGBKT7 bait vector (GAL4-binding domain; DBD). *ASK1*, *PslGAI*, *PslRGL*, and *PslRGA* cDNAs were fused into the *Nde*I–*Bam*HI site of the pGADT7 prey vector (GAL4 activation domain; AD). The resuspended yeast were spread in 96-well plates containing DDO/X/A medium in the presence or absence of 100 μM GA_3.

Bimolecular fluorescence complementation (BiFC) assay

Full-length and truncated *PslSLY1* ORFs were fused into the *Sal*I–*Bam*HI site of the pSAT1-*N* vector, containing N-terminal YFP (NY). *ASK1* and *PslRGL* sequences were cloned into the *Sac*II–*Bam*HI site of the pSAT1-*C* vector, containing C-terminal YFP (CY). The different combinations of constructs encoding NY and CY at similar concentrations were mixed and then co-transfected into protoplasts obtained from suspension-cultured tobacco *BY-2* cells, as previously described (El-Sharkawy *et al.*, 2014*a*). Empty BiFC vectors (NY: gene/CY and NY/CY: gene) were used as negative controls. Protoplasts were analyzed for fluorescence by confocal microscopy.

Plant transformation

Full-length and truncated *PslSLY1* ORFs were fused into the *Spe*I–*Bst*BI site of the pCambia1305.1 binary vector and expressed under the control of the 35S promoter. The constructed vectors were transformed into *Agrobacterium tumefaciens* and introduced into *Arabidopsis* as described previously (El-Sharkawy *et al.*, 2012). The full-length *PslSLY1* ORF was introduced into the *Arabidopsis* WT L*er* background; however, the full-length and truncated *PslSLY1* ORFs were introduced into the *Arabidopsis* mutant *sly1.10* (McGinnis *et al.*, 2003). T_3 homozygous independent lines from each group were grown (24 plants/group/growth condition) under long-day (LD) conditions (16:8 h light/300 μmol m^{-2} s^{-1}, 23 °C:18 °C, and 65% relative humidity) and treated or not treated with PAC (5 μM). Plant materials were frozen in liquid N_2 immediately after collection and stored at −80 °C until use.

Accession numbers

The sequence data used in this study can be found in the GenBank database under the following accession numbers: PslSLY1 (MT358313),

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gPslSLY1 (MT358314), PslGAI (KU845589), PslRGL (KU845592), and ASK1 (U70034).

Results and discussion

In silico *characterization of PslSLY1*

In line with our previous research into the factors regulating stone fruit growth (El-Sharkawy *et al.*, 2012, 2014*a*, 2017), this study was conducted to elucidate the function of an SLY1 F-box protein in the plum cultivar, EG. The isolated gene, *PslSLY1*, contains an ORF of 598 bp encoding a protein of 186 amino acids with a calculated molecular mass of 20.7 kDa and an isoelectric point of 8.44. The genomic *gPslSLY1* sequence is 2159 bp in length, comprising a 1245 bp promoter sequence that carries several predicted GA and auxin *cis*-acting regulatory elements presumed to be involved in the regulation of *PslSLY1* transcription (Supplementary Table S2). As with the majority of previously identified *SLY1/GID2* gene family members (McGinnis *et al.*, 2003; Dill *et al.*, 2004), the plum gene is encoded by an intronless ORF. One exception is the hexaploid wheat, where the *TaGID2* genes display two exons interrupted by one intron (Lou *et al.*, 2016). Most characterized plants have only one GA-specific F-box protein that belongs to the *SLY1/GID2* subfamily (McGinnis *et al.*, 2003; Sasaki *et al.*, 2003); however, recent studies have demonstrated the presence of two biologically functional SLY1/GID2 paralogs in grape (Acheampong *et al.*, 2015). Sequence data mining in the genomes of *Prunus* species, including *P. persica*, *P. avium*, *P. armeniaca*, *P. yedoensis*, and *P. dulcis* (https://www.rosaceae.org), indicated that all *Prunus* species have only one *SLY1/GID2* homolog. One exception is the ornamental flowering cherry (*P. yedoensis*) that has two putative paralogs.

Multiple alignments of *PslSLY1* with other reported *SLY1/GID2* sequences from different species indicated a high sequence divergence (32–87% similarity), largely due to extensive deviation in the N- and C-terminal domains outside the typical F-box region (Fig. 1). However, several signature structural elements commonly associated with the F-box subunit protein of SCF ubiquitin ligases were identified (McGinnis *et al.*, 2003; Sasaki *et al.*, 2003). The predicted amino acid sequence of PslSLY1 begins with an N-terminal domain of unknown function that displays striking sequence dissimilarity, in terms of length (61–94 amino acids in monocots and 32–48 amino acids in dicots) and homology (37–70% and 26–72% similarity in monocots and dicots, respectively), to other SLY1-like proteins. This is followed by the distinctive F-box sequence (46 amino acids) that has a greater sequence similarity (63–100%) to other SLY1 homologs. Finally, similarly to the N-terminal region, the C-terminal region displays a substantial variability in its predicted protein length (73–134 amino acids) and homology (29–86% similarity) to other SLY1-like proteins. The C-terminal region, however, has two short conserved domains, GGF (33–37 amino acids; 56–91% similarity) and LSL (26 amino acids; 66–100% similarity) that suggest functionality for this domain.

The phylogenetic tree separated the GA F-box proteins into two subfamilies, SLY1/GID2 and SNE. The SLY1/GID2 subfamily can be further divided into two classes based on angiosperm monocots and eudicots (Supplementary Fig. S1). PslSLY1 has strong sequence identity to SLY1/GID2 proteins from other members of the family Rosaceae. The relationship was more distant with *Arabidopsis*, grape, and tomato SLY1/GID2 homologs, indicating that the evolutionary relationship is consistent with taxonomic distance.

Characterization of PslSLY1 domains

All SLY1/GID2 genes are highly conserved, with predicted proteins that maintain three typical domains generally associated with the F-box subunit of the SCF E3 ubiquitin ligase complex (Fig. 1). To determine the role of each domain in the protein function, we investigated the subcellular and biochemical characteristics of full-length ORFs and ORFs displaying a deletion in each of the three PslSLY1 domains. The complete protein is referred to as PslSLY1. PslSLY1 protein lacking

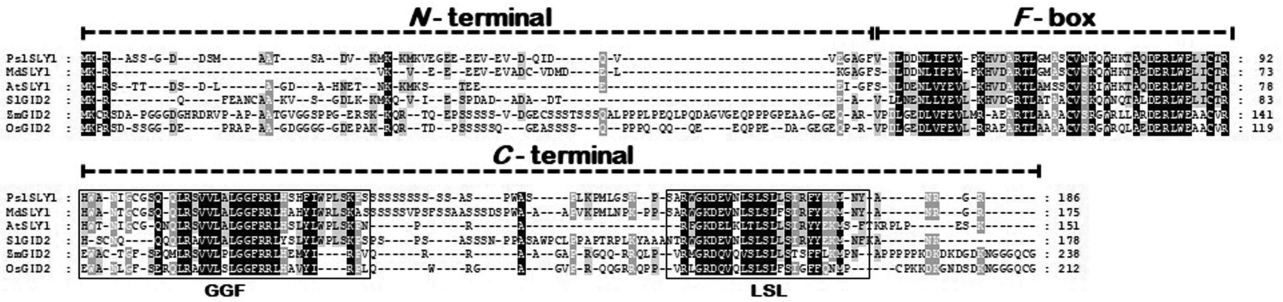

Fig. 1. Amino acid sequence alignment of *PslSLY1* with closely related sequences from *M. domestica*, *A. thaliana*, *S. lycopersicum*, *Z. mays*, and *O. sativa*, using ClustalX. Conserved residues are shaded in black. Dark gray shading indicates similar residues in six out of seven of the sequences, and light gray shading indicates similar residues in at least four of the sequences. The three main domains, N-terminus, F-box, and C-terminus are indicated, and the conserved GGF and LSL motifs are outlined.

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the N-terminal, F-box, or C-terminal domains are labeled PslSLY1$_{\Delta N}$, PslSLY1$_{\Delta F}$, or PslSLY1$_{\Delta C}$, respectively.

Subcellular localization of PslSLY1 and its truncated derivatives

An *in silico* analysis of the amino acid sequences of the *SLY1/GID2* family failed to identify any motifs that can signify the localization of the protein in the plant cell. The SignalP Server, for instance, predicted that the PslSLY1 is a non-transmembrane, cytoplasmic protein. Fluorescence microscopy illustrated that control tobacco *BY-2* cells transformed with the GFP gene alone showed ubiquitous fluorescence, following the predicted cytosolic localization of the GFP proteins (Fig. 2A). However, a full-length PslSLY1–GFP fusion protein localized exclusively to the nucleus (Fig. 2B), suggesting that PslSLY1 is capable of redirecting the GFP signal from the cytosol to the nucleus. This is consistent with its primary function as a component of the SCF E3 ubiquitin ligase complex that targets the DELLA proteins for degradation within the plant cell nucleus (Dill *et al.*, 2004). To investigate the structural role of PslSLY1 domains, we explored the basis of localization of PslSLY1 proteins using independent domain removal. The elimination of the N-terminal domain did not affect the nuclear location of PslSLY1$_{\Delta N}$ protein (Fig. 2C). In contrast, the eradication of the F-box and C-terminal domain produced a dramatic disruption of protein localization. In PslSLY1$_{\Delta F}$–GFP chimeras, a small proportion of the signal was visualized in the nucleus or cytoplasm; however, the majority of the chimeric GFP protein was detected on the exterior side of the nuclear membrane (Fig. 2D). Likewise, removal of the C-terminal domain in the PslSLY1$_{\Delta C}$ derivative noticeably altered the protein localization pattern (Fig. 2E). Without the C-terminus, the protein was no longer confined to the nucleus, as the GFP signal consistently covered the cytoplasm and nucleus. Treatment with GA before transfection did not alter the localization of the different PslSLY1 versions. These results indicate the fundamental role played by elements included in the F-box and C-terminal domain in ensuring appropriate localization of the PslSLY1 protein.

PslSLY1 is a component of an SCF complex that targets DELLA proteins

F-box subunits are assembled into an SCF complex (Zhang *et al.*, 2019). If the PslSLY1 protein is part of a functional SCF^{PslSLY1} complex, it should interact with downstream proteins involved in GA signaling (Dill *et al.*, 2004; Fu *et al.*, 2004). We tested the ability of PsISLY1 to interact with the *Arabidopsis* Skp1 protein (ASK1) using the Y2H system in GA-free or GA-containing media. Consistent with SLY1/GID2 F-box proteins, PslSLY1 was effectively capable of interacting with ASK1. The binding results suggested that GA is not essential for establishing a stable SCF^{PslSLY1} complex in yeast (Fig. 3A).

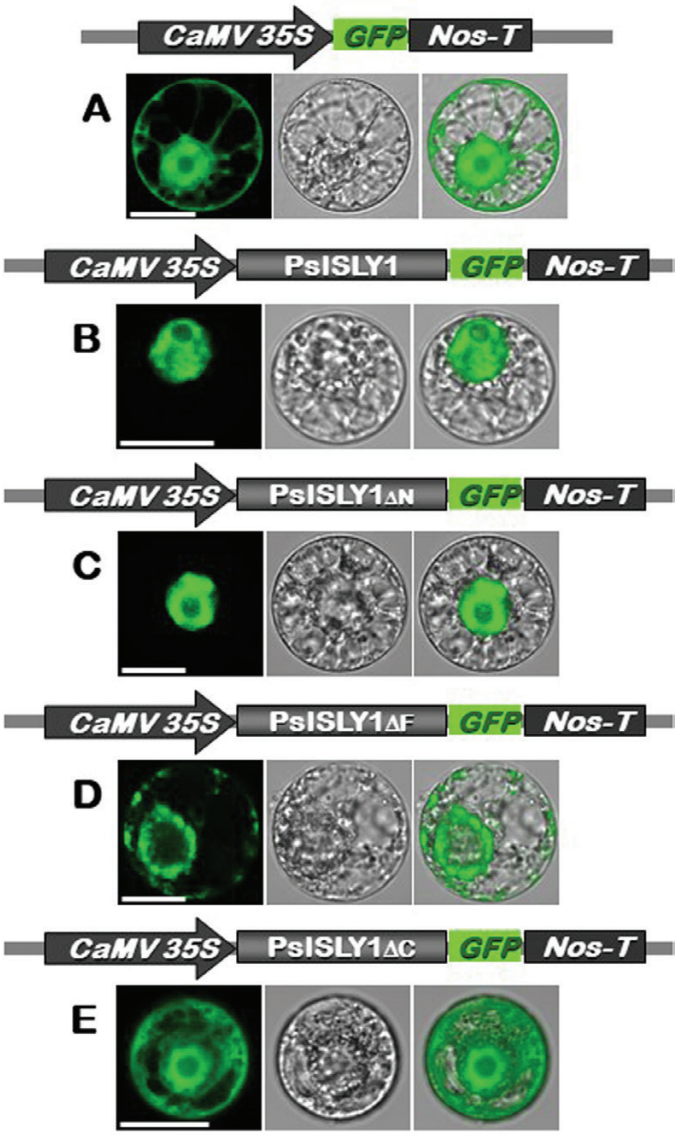

Fig. 2. Subcellular localization of PslSLY1 and truncated proteins. The full-length ORF is referred to as PslSLY1. Truncated derivatives, including independent deletions of the N-terminal, F-box, and C-terminal domains, are referred to as PslSLY1$_{\Delta N}$, PslSLY1$_{\Delta F}$, and PslSLY1$_{\Delta C}$, respectively. Constructs of either (A) GFP as control, (B) PslSLY1–GFP, (C) PslSLY1$_{\Delta N}$–GFP, (D) PslSLY1$_{\Delta F}$–GFP, or (E) PslSLY1$_{\Delta C}$–GFP were transiently transformed into *Nicotiana tabacum* protoplasts. GFP fluorescence was visualized using confocal laser scanning microscopy. Fluorescence (left panel) and light micrograph (center panel) images are merged (right panel) to illustrate the different locations of the two proteins. Scale bar=10 μm. (This figure is available in color at *JXB* online.)

We further assessed the interaction properties of PslSLY1 with DELLA repressors to define whether DELLA proteins are prospective substrates of the SCF^{PslSLY1} E3 ubiquitin ligase. The well-characterized DELLA proteins selected for this experiment were the plum PslGAI, PslRGL, and PslRGA that represent three subclades of DELLA proteins and play overlapping

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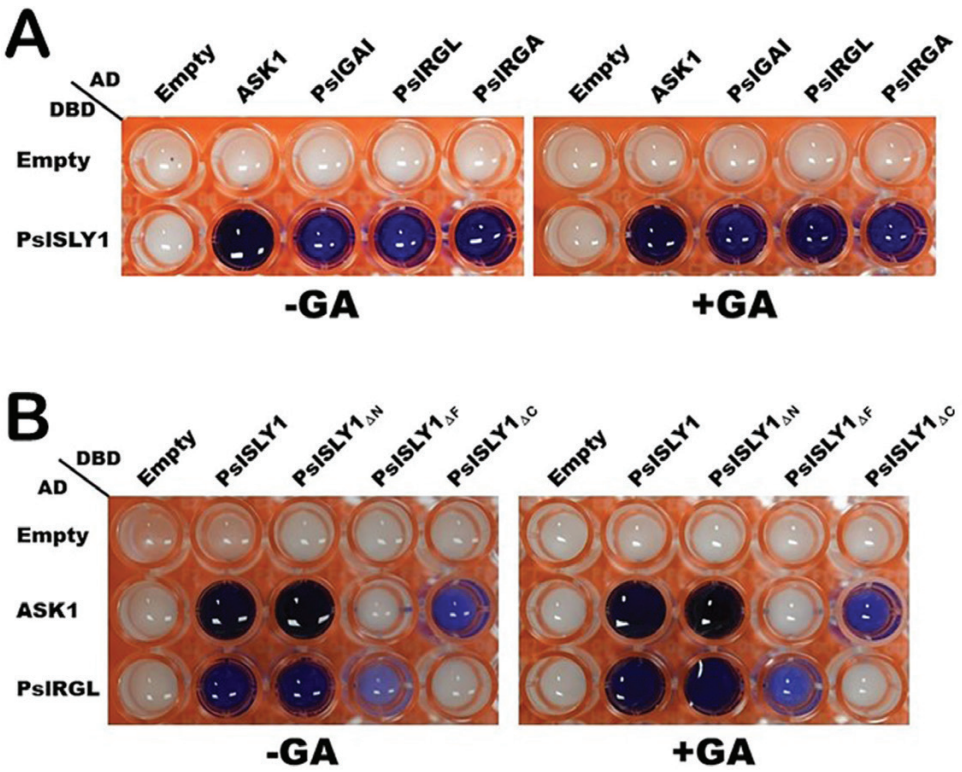

Fig. 3. (A) Differences in interactions between PslSLY1–ASK1 and –PslDELLAs, and (B) PslSLY1 truncated derivatives (PslSLY1$_{\Delta N}$, PslSLY1$_{\Delta F}$, and PslSLY1$_{\Delta C}$) with ASK1 and PslRGL using a yeast system. Y2H assays of the interactions were performed using PslSLY1 or derivatives as bait in yeast strain Y2HGold and ASK1 or PslDELLAs as prey in the Y187 strain. The mated yeast were grown in 96-well plates containing DDO/X/A selective medium in the presence or absence of 100 μM GA$_3$. All experiments were repeated at least three times. (This figure is available in color at *JXB* online.)

and distinct roles in mediating GA responses during plum fruit development (El-Sharkawy *et al.*, 2017). The analysis revealed that PslSLY1 had an analogous binding capacity in a GA-independent manner for each of the PslDELLAs (Fig. 3A).

To verify the role of each PslSLY1 domain in the binding selectivity of the diverse interacting proteins, the truncated *PslSLY1* ORFs were tested for interaction with ASK1 and PslRGL in the presence or absence of GA using the Y2H system (Fig. 3B). Relative to the full-length control PslSLY1, the exclusion of the N-terminal domain did not strikingly disrupt the GA-independent interaction capacity of PslSLY1$_{\Delta N}$ with ASK1 or PslRGL. In contrast, the deletion of either the F-box or the C-terminal domains altered protein interactions. The omission of the F-box domain hindered the interaction between the PslSLY1$_{\Delta F}$ protein and ASK1, and diminished the capacity for interaction with PslRGL. The PslSLY1$_{\Delta C}$ ORF was able to interact with ASK1, although at considerably weaker levels; however, the lack of the C-terminal domain inhibited the interaction with PslRGL. To provide further evidence for yeast results, we visualized the interaction pattern of different PslSLY1 versions using the BiFC approach (Fig. 4). The YFP fluorescence signal triggered by interactions with ASK1 was only detected with PslSLY1, PslSLY1$_{\Delta N}$, and PslSLY1$_{\Delta C}$ derivatives. Fluorescence with PslRGL was only observed with the PslSLY1, PslSLY1$_{\Delta N}$, and PslSLY1$_{\Delta F}$ derivatives. It has been shown that the F-box domain functions as a protein–protein interaction site during the establishment of the SCF E3 ubiquitin ligase complex and the C-terminal domain is essential for binding to the DELLA repressor substrate (Dill *et al.*, 2004; Fu *et al.*, 2004; Gomi *et al.*, 2004; Ariizumi *et al.*, 2011). The data outlined above demonstrate that PslSLY1 is a functional F-box component that can form an SCF^{PslSLY1} ubiquitin ligase complex and putatively target DELLA proteins for degradation. The loss of the N-terminal domain had minor consequences for ligand–complex formation and function.

GAs coordinate a wide range of systems through various response regulation mechanisms, including biosynthesis, metabolism, and signaling (Yasumura *et al.*, 2007; Yamaguchi, 2008). *In planta*, GID1 receptors and DELLA repressors interact via conserved N-terminal DELLA regulatory domains after GID1 binding to the active GA. The formation of the GA–GID1–DELLA co-receptor complex leads to conformational changes in the DELLA protein that facilitates recognition between the GRAS domain of the DELLA protein and the SLY1/GID2 C-terminal domain (Dill *et al.*, 2004; Fu *et al.*, 2004; Hirano *et al.*, 2010; Ariizumi *et al.*, 2011; Lou *et al.*, 2016). The DELLA proteins are subsequently degraded via the SCF^{PslSLY1} ubiquitin-dependent proteolysis pathway. Although

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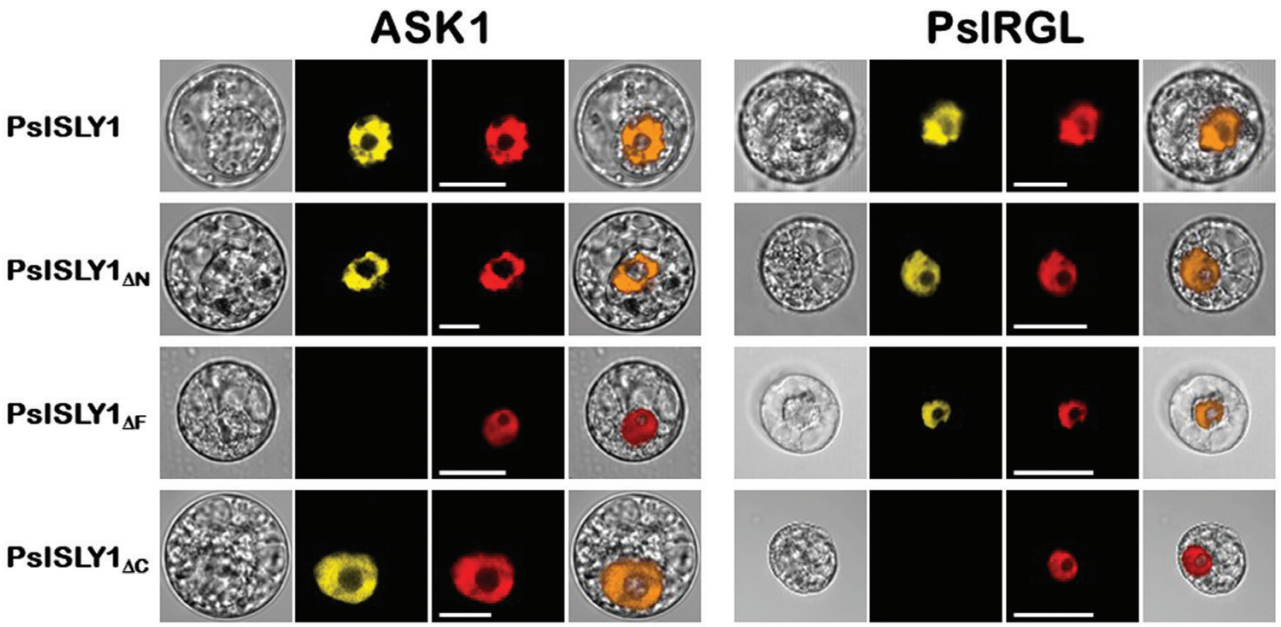

Fig. 4. BiFC visualization of PslSLY1 and truncated derivative (PslSLY1$_{\Delta N}$, PslSLY1$_{\Delta F}$, and PslSLY1$_{\Delta C}$) interactions with ASK1 and PslRGL. PslSLY1 and its derivatives were fused with the N-terminus (NY) of YFP. ASK1 and PslRGL were fused with the C-terminus (CY) of YFP. Different combinations of NY and CY constructs were transiently co-expressed in tobacco protoplasts. NLS–mCherry was included in each transfection to highlight the location of the nucleus. The merged image is a digital merge of bright field and fluorescent images to illustrate the interaction location. Scale bars=10 μm. (This figure is available in color at *JXB* online.)

this mechanism is widespread, studies have demonstrated an alternative mechanism via a GA–GID1–DELLA co-receptor, proteolysis-independent pathway (Ariizumi *et al.*, 2008, 2013).

Among the three tested PslDELLA proteins, PslRGA represents a new branch of DELLA protein evolution, with no representative in *Arabidopsis* (El-Sharkawy *et al.*, 2017). PslRGA displays considerable divergence in the essential N-terminal DELLA regulatory domain, including the key DELLA, LExLE, and VHYNP motifs important for GA-dependent GID1–DELLA protein interactions. The lack of a conserved DELLA regulatory domain restricted GA–GID1–DELLA interactions in the Y2H system (Griffiths *et al.*, 2006; Hirano *et al.*, 2010; El-Sharkawy *et al.*, 2017). PslRGA also appears to encode a GA-insensitive DELLA protein since PslRGA−GFP fluorescence showed resistance to the destabilizing impact of GA in *Arabidopsis* roots (El-Sharkawy *et al.*, 2017). Despite this, the GRAS domain containing PslRGA displayed a strong capacity for interacting with PslSLY1 *in vitro*. It appears that the first step of GA–GID1–DELLA co-receptor complex assembly is imperative for the transition to DELLA suppression via the proteolysis-dependent degradation pathway.

PslSLY1 *expression during fruit ontogeny*

SLY1/GID2 family members have been reported to be differentially regulated in different tissues (Acheampong *et al.*, 2015; Liu *et al.*, 2016; Lou *et al.*, 2016). *PslSLY1* mRNA accumulation varied in an organ- and developmental stage-dependent manner (Fig. 5). This suggests that *PslSLY1* is transcriptionally regulated, as proposed for its ortholog in *Arabidopsis* (McGinnis *et al.*, 2003). *PslSLY1* transcripts were moderately abundant in flower buds (~4 d before bloom) followed by a marked decline at full bloom. After fertilization, *PslSLY1* mRNA peaked at ~7 days after bloom (DAB) and then declined during early fruit formation (~10–22 DAB). GA plays a role in diverse biological and physiological processes, especially flowering and fruit initiation (Blázquez *et al.*, 1998; Goto and Pharis, 1999; Vivian-Smith and Koltunow, 1999; Davies, 2004; Bernier and Perilleux, 2005). It is also involved in coordinating the cell division, expansion, and embryo development during the fruit set phase (Serrani *et al.*, 2007; de Jong *et al.*, 2009, El-Sharkawy *et al.*, 2014*a*). The accumulation profile of *PslSLY1* transcripts in floral buds and directly after fertilization indicates a role in floral meristem development and flower bud initiation, as well as in the GA regulation of the flower-to-fruit transition program.

Stone fruit (*Prunus* spp.), including plums, display a characteristic double sigmoid growth pattern during fruit development, with four distinct stages, S1–S4 (El-Sharkawy *et al.*, 2014*a*). The *PslSLY1* transcription profile suggested its contribution to the GA signaling network that modulates GA responses, regulating fruit growth during the early S1–S2 stages of fruit development. *PslSLY1* mRNA declined gradually from early S3 stage (~57 DAB) throughout the remainder of fruit development, reaching very low levels by the post-climacteric phase (~83 DAB). In plum, transcript profiles of GA-related

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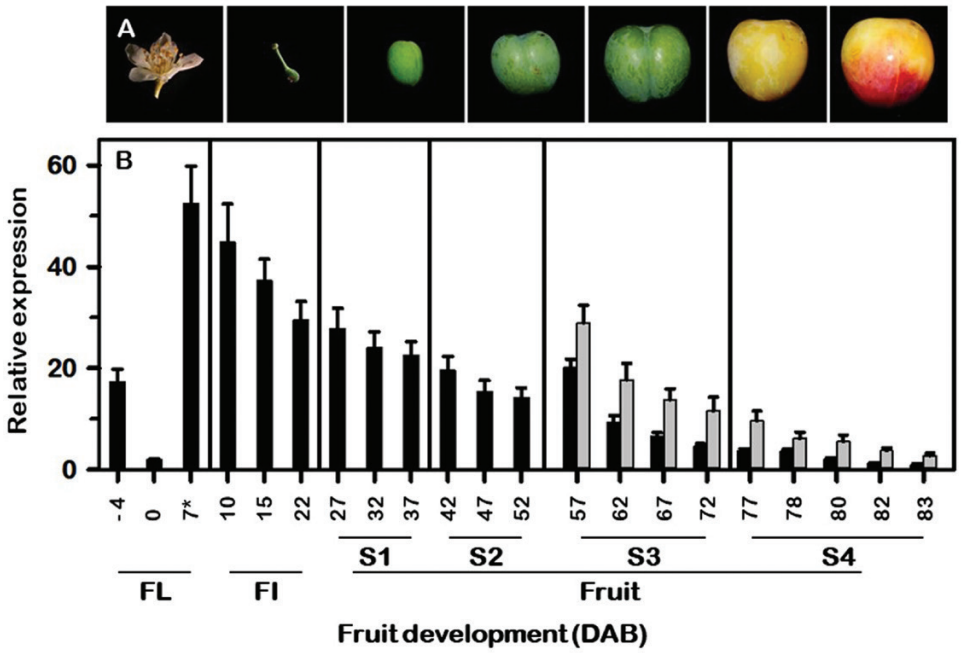

Fig. 5. Steady-state transcript levels of *PslSLY1* mRNA assessed by qPCR in EG flowers and fruit throughout development. Expression was determined in the whole fruit during stages S1 and S2 of fruit development. During S3 and S4, expression was determined in pulp (black filled bars) and in seeds (gray filled bars). Transcript accumulation was determined using qPCR on three biological replicates. Error bars represent the SD of the mean. Standard curves were used to calculate the number of molecules of the target gene per sample, which were then normalized relative to *PslAct* expression. The *y*-axis refers to the relative expression. The *x*-axis represents the developmental stages indicated by the number of days after bloom (DAB). (This figure is available in color at *JXB* online.)

genes are usually aligned with their GA regulation model. When GA is abundant, the GA proteins that act as activators (i.e. GA3ox, GA20ox, GID1, and SLY1/GID2) and repressors (i.e. GA2ox and DELLA) of GA action are subject to negative feedback and positive feed-forward regulation by GA, respectively (Griffiths *et al.*, 2006; Yasumura *et al.*, 2007; Yamaguchi, 2008; Harberd *et al.*, 2009). The accumulation pattern of bioactive GAs during plum fruit development (i.e. in the same developmental stages adopted in the current study) proposed that both GA_1 and GA_4 are directly implicated in S3 events via elevating the rate of cell expansion and increasing fruit size (El-Sharkawy *et al.*, 2014*a*). The data indicate that *PslSLY1* is an element of the GA signaling network during the S3 stage of fruit development and during the ripening phase (S4) in a distinctive GA-dependent manner. This argument is supported by high levels of GA_1 detected through ripening (El-Sharkawy *et al.*, 2014*a*).

GA regulation of PslSLY1

The abundance of *PslSLY1* mRNA in organs with low levels of GA might be due to feedback inhibition of expression of the gene during dynamic GA signaling. To examine this, *PslSLY1* mRNA accumulation profiles were evaluated in fruits supplemented with GA or the GA biosynthesis inhibitor PAC. GA treatment markedly reduced *PslSLY1* mRNA levels. Compared with the controls, *PslSLY1* mRNA levels declined by ~74% in GA-treated samples collected 9 d after treatment (Supplementary Fig. S2a). In contrast, treatment with PAC steadily increased *PslSLY1* transcript abundance to ~5-fold over the same treatment period (Supplementary Fig. S2b). These results suggest that in plum, the negative regulation of *PslSLY1* mRNA abundance is part of the GA response in plum.

In planta *function of PslSLY1*

The results confirmed that PslSLY1 could act as an effective F-box protein that is able to interact with the ASK1 subunit of an SCF–ubiquitin ligase complex and potentially targeting PslDELLA repressors for ubiquitination in a GA-independent manner. In *Arabidopsis* and other species, it has been shown that GA relieves growth repression by promoting interactions between GID1s and DELLAs, triggering the ubiquitin-dependent proteolysis of the DELLA proteins via the $SCF^{SLY1/GID2}$ and the 26S proteasome (Yasumura *et al.*, 2007; Ariizumi *et al.*, 2008).

To test whether PslSLY1 protein maintains similar GA functions *in planta*, several independent *Arabidopsis* transgenic lines overexpressing *PslSLY1* (SLY1/w) were generated. Plants from two independent T_3 homozygous lines (L2 and L9) were chosen for further analysis (Fig. 6A). Theoretically, ectopically expressing *PslSLY1* should

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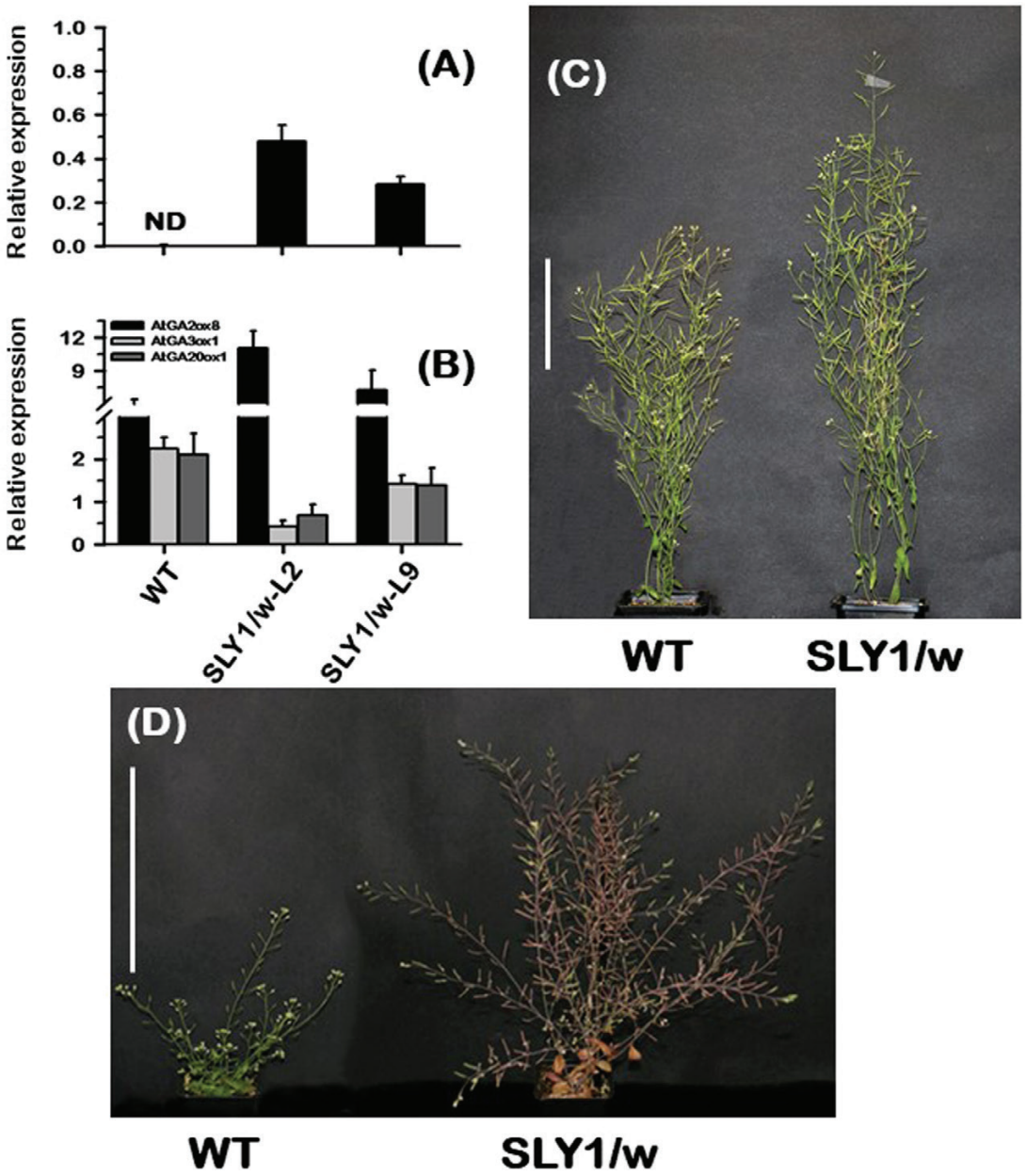

Fig. 6. (A) *PslSLY1* transgene accumulation and (B) accumulation levels of the GA-related mRNAs in WT and two transgenic lines (*SLY1*/w-L2 and *SLY1*/w-L9), overexpressing the *PslSLY1* sequence. Transcript accumulation was determined using qPCR on three biological replicates. Error bars represent the SD of the mean. Standard curves were used to calculate the number of molecules of the target gene per sample, which were then normalized relative to *AtAct* expression. The *y*-axis refers to the relative expression. The *x*-axis represents the *Arabidopsis* plants. ND, non-detectable. Aerial portions of the WT and *SLY1*/w-L2 transgenic mutant grown under LD conditions/non-treated (C) or /treated (D) with PAC (5 μM). Scale bars=10 cm.

accelerate the GA responses and trigger overall plant growth. Accordingly, SLY1/w transgenic plants were phenotypically characterized for a range of GA-related traits. As expected, overexpression of *PslSLY1* in WT *Arabidopsis* produced dramatic phenotypic changes consistent with elevated GA responsiveness. The phenotypic variations from the WT became more distinguishable in plants treated with the GA biosynthesis inhibitor PAC. The accumulation of transcripts involved in GA metabolism were assessed, including genes encoding enzymes for GA biosynthesis (*GA3ox* and *GA20ox*) and enzymes for GA inactivation (*GA2ox*) (Thomas *et al.*, 1999; Xu *et al.*, 1999; Sponsel and Hedden, 2004; Sun and Gubler, 2004). Consistent with the GA regulation model (Yamaguchi, 2008), the accumulation of *AtGA3ox1* and *AtGA20ox1* was dramatically reduced by ~61% and ~51% relative to the WT, respectively, while *AtGA2ox8* steadily increased by ~2-fold in SLY1/w plants (Fig. 6B). Overexpression of *PslSLY1* in WT *Arabidopsis* led

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Table 1. Vegetative and reproductive characteristics of WT (L*er*) *Arabidopsis* plants overexpressing the *PslSLY1* gene as shown in Fig. 6

Character	Condition	WT	SLY1/w
Germination rate (%)	C	90.0±1.9	98.0±0.8**
Germination time (d)	C	2.7±0.4	2.4±0.2^{ns}
Stem height (cm)[a]	C	32.7±1.5	44.5±2.2**
	PAC	8.8±0.5	22.4±1.9**
Internode length (cm)[a,b]	C	2.4±0.2	3.0±0.3*
	PAC	0.6±0.3	1.4±0.2**
No. of internodes[a]	C	13.4±0.2	14.0±0.3*
	PAC	14.3±0.4	16.5±0.2**
Transition to flowering (d)[c]	C	28.3±0.6	26.2±0.8*
	PAC	45.3±1	36.6±0.9**
Pistil length (mm)[c,d]	C	2.21±0.08	2.55±0.09**
	PAC	1.52±0.07	1.98±0.06**
Stamen length (mm)[c,d]	C	2.16±0.04	2.39±0.07**
	PAC	1.26±0.08	1.78±0.09**
Silique initiation time (d)[e]	C	2.4±0.2	2.1±0.2^{ns}
	PAC	3.7±0.3	2.8±0.3*
Silique maturation time (d)[a]	C	13±0.6	9.2±0.4**
	PAC	21.4±0.9	12.1±0.7**
Silique length (mm)[e,f]	C	10.29±0.11	12.59±0.2**
	PAC	4.56±0.07	8.52±0.12**
No. of seeds/silique [e,f]	C	42.7±3.2	46.3±2.6^{ns}
	PAC	13.0±1.7	29.8±3.3**

Plants were grown under an LD photoperiod and divided into two groups: control and treated with PAC (5 μM). The measurements are the means (±SD) of ~12 plants. Statistically significant differences from the WT are indicated by * and ** for the probability levels $P<0.05$ and $P<0.01$, respectively. ns, non-significant ($P>0.05$).

[a] The measurements were taken from adult plants and have ~10% shattered siliques.

[b] The first vegetative internode in the main inflorescence.

[c] Flowering time was scored upon the emergence of ~10% of flowers.

[d] The measurements are the means (±SD) of 12 flowers.

[e] The measurements were scored upon the emergence of 10% of siliques.

[f] The measurements are the means (±SD) of 12 siliques.

to extensive disturbances in overall growth and development consistent with altered GA signaling (Fig. 6C). Analyses of the transgene in several transgenic lines showed that the level of *PslSLY1* mRNA was usually correlated with the strength of the phenotype (i.e. the higher the level of expression of the transgene the more pronounced the effect on growth). SLY1/w plants manifested significantly higher germination rates, enhanced vegetative growth associated with increases in the number and length of internodes, precocious transition to flowering, longer pistil/stamen lengths, and larger siliques with earlier silique dehiscence (Table 1). Treatment of the SLY1/w plants with PAC (Fig. 6D) demonstrated that ectopic *PslSLY1* expression enhanced the resistance to the growth impact of PAC, even for traits that presented non-significant contrasts with WT plants under optimal conditions (Table 1).

Overexpression of PslSLY1 *rescues the* sly1.10 *mutant*

To further verify that *PslSLY1* can have biologically similar functions to its orthologs, we tested its capability to overcome the loss of function of the endogenous AtSLY1 in the GA-insensitive *sly1.10* mutant (McGinnis *et al.*, 2003). Truncated *PslSLY1* derivatives were introduced into the *sly1.10* loss-of-function mutant line to define the strength of the GA response under the independent loss of the major PslSLY1 domains. The different PslSLY1 lines were phenotypically characterized under standard growth conditions.

Several biochemical and molecular studies illustrated the crucial role played by GA in coordinating the germination rate and the time necessary to establish germination (Lee *et al.*, 2002; Tyler *et al.*, 2004; Ariizumi *et al.*, 2008, 2011). The *sly1.10* mutant has a prolonged seed dormancy and germinates at a much slower rate (Ariizumi and Steber, 2007). The germination of seeds resulting from *sly1.10* plants overexpressing *PslSLY1*$_{\Delta F}$ (SLY1$_{\Delta F}$/s) and *PslSLY1*$_{\Delta C}$ (SLY1$_{\Delta C}$/s) behaved similarly to the *sly1.10* mutant, via displaying poor germination rates and duration, either with or without the addition of exogenous GA (Table 2; Supplementary Fig. S3). In contrast, the introduction of *PslSLY1* or *PslSLY1*$_{\Delta N}$ ORFs restored WT germination efficiencies in the transgenic plants.

Several independent transgenic events were produced and one representative from each transformation, conferring a maximum transgene level, was selected for further studies (Fig. 7A). To estimate whether overexpression of *PslSLY1* and its derivatives can recover the disrupted GA responsiveness in the mutant, the accumulation of GA metabolism genes was assessed in controls (WT and *sly1.10* mutant) and transformed plants. Relative to the *sly1.10* mutant line, *sly1.10* plants transformed with *PslSLY1*$_{\Delta F}$ (SLY1$_{\Delta F}$/s) and *PslSLY1*$_{\Delta C}$ (SLY1$_{\Delta C}$/s) derivatives showed no meaningful differences in the transcription levels of the three genes involved in GA metabolism (Fig. 7B). However, ectopic expression of the full-length *PslSLY1* (SLY1/s) and the *PslSLY1*$_{\Delta N}$ (SLY1$_{\Delta N}$/s) led to a dramatic restoration of the GA metabolic pathway. It remarkably reduced transcript levels of *AtGA3ox1* by ~89% and ~82%, and of *AtGA20ox1* by ~87% and ~82%, respectively. In contrast, *AtGA2ox8* mRNA increased by ~20- and ~18-fold in SLY1/s and SLY1$_{\Delta N}$/s plants, respectively.

The *sly1.10* mutant plants have reduced growth compared with WT plants (Table 2). Consistent with the results obtained from subcellular localization and protein–protein interactions, *PslSLY1*$_{\Delta F}$ and *PslSLY1*$_{\Delta C}$ plants displayed phenotypes consistent with the repression of GA action, similar to the *sly1.10* mutant phenotype (Table 2; Fig. 7C). These results validated that the F-box and C-terminal domains are vital for proper SLY1/GID2 protein function (Dill *et al.*, 2004; Fu *et al.*, 2004; Gomi *et al.*, 2004; Ariizumi *et al.*, 2011). Introducing *PslSLY1* and *PslSLY1*$_{\Delta N}$ ORFs into *sly1.10* visibly restored growth via re-establishing the typical plant height, mainly due to

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Table 2. Vegetative and reproductive characteristics of *Arabidopsis* WT (L*er*), *sly1.10* mutant, and *sly1.10* plants overexpressing *PslSLY1*, *PslSLY1*$_{\Delta N}$, *PslSLY1*$_{\Delta F}$, and *PslSLY1*$_{\Delta C}$ ORFs as shown in Fig. 7, and Supplementary Figs S3 and S4.

Character	*sly1.10*	SLY1/s	SLY1$_{\Delta N}$/s	SLY1$_{\Delta F}$/s	SLY1$_{\Delta C}$/s	WT
Germination rate (%)	11.5±2.5	80.7±1.4**	83.2±1.9**	9.1±1.1 ^{NS}	7.9±1.6^{ns}	90.0±1.9
Germination time (d)	21.9±0.3	3.0±0.2**	2.9±0.3**	23.3±0.3^{ns}	20.0±0.2^{ns}	2.7±0.4
Stem height (cm)[a]	5.7±0.3	27.8±1.3**	26.4±0.8**	6.1±0.4^{ns}	5.5±0.5^{ns}	32.7±1.5
Internode length (cm)[a,b]	0.8±0.2	2.1±0.4**	2.0±0.7**	0.8±0.3^{ns}	0.7±0.1^{ns}	2.4±0.2
No. of internodes[a]	7.5±0.4	13.3±0.3**	13.1±0.4**	7.4±0.3^{ns}	7.8±0.6^{ns}	13.4±0.2
Transition to flowering (d)[c]	47.7±1.2	29.2±0.9**	30.1±1.1**	50.0±1.7^{ns}	49.2±0.9^{ns}	28.3±0.9
No. of flowers/inflorescence	16.0±1.6	48.4±5.5**	49.5±5.4**	15.1±1.5^{ns}	17.2±2.2^{ns}	52.3±10.5
Pistil length (mm)[c,d]	1.00±0.05	2.22±0.13**	2.19±0.09**	0.90±0.07^{ns}	1.11±0.06^{ns}	2.21±0.08
Stamen length (mm)[c,d]	0.92±0.03	2.15±0.05**	2.11±0.08**	0.84±0.05^{ns}	0.95±0.02^{ns}	2.16±0.04
Silique initiation time (d)[e]	3.9±0.5	2.5±0.2**	2.7±0.3**	4.1±0.6^{ns}	3.7±0.4^{ns}	2.4±0.2
Silique maturation time (d)[a]	29.0±1.6	13.7±0.9**	13.3±0.7**	31.3±1.0^{ns}	30.8±0.9^{ns}	13±0.6
Silique length (mm)[e,f]	3.91±0.06	9.96±0.15**	9.67±0.17**	4.06±0.08^{ns}	3.93±0.09^{ns}	10.29±0.11
No. of seeds/silique[e,f]	9.8±1.5	39.7±1.6**	40.1±2.7**	10.2±1.2^{ns}	9.9±1.3^{ns}	42.7±3.2

Plants were grown under an LD photoperiod and divided into two groups: control and treated with PAC (5 μM). The measurements are the means (±SD) of ~12 plants. Statistically significant differences from the WT are indicated by * and ** for the probability levels $P<0.05$ and $P<0.01$, respectively. ns, non-significant ($P>0.05$).

[a] The measurements were taken from adult plantsand have ~10% shattered siliques.

[b] The first vegetative internode in the main inflorescence.

[c] Flowering time was scored upon the emergence of ~10% of flowers.

[d] The measurements are the means (±SD) of 12 flowers.

[e] The measurements were scored upon the emergence of 10% of siliques.

[f] The measurements are the means (±SD) of 12 siliques.

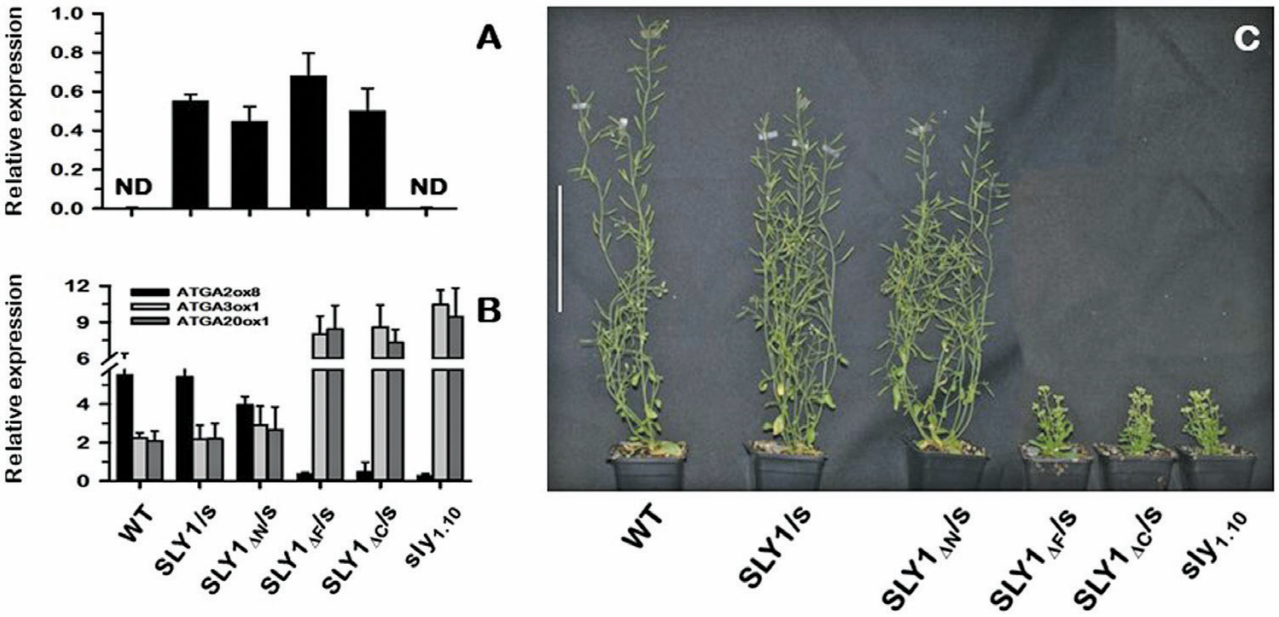

Fig. 7. (A) Transgene accumulation and (B) expression levels of the different GA-related mRNAs in the WT, *sly1.10* mutant, and *sly1.10* plants overexpressing *PslSLY1* (SLY1/s) and *PslSLY1*$_{\Delta N}$ (SLY1$_{\Delta N}$/s), *PslSLY1*$_{\Delta F}$ (SLY1$_{\Delta F}$/s) and *PslSLY1*$_{\Delta C}$ (SLY1$_{\Delta C}$/s). Transcript accumulation was determined using qPCR on three biological replicates. Other details are as described in Fig. 6. (C) Aerial portions of *sly1.10* mutant and the transgenic lines were grown under LD (16 h day/8 h night) conditions. ND, non-detectable. Scale bars=10 cm.

enhancements in internode length and number. Relative to the control *sly1.10* mutant, SLY1/s and SLY1$_{\Delta N}$/s plants demonstrated significant enrichment in their overall heights by ~5- and ~4.8-fold, respectively (Table 2; Fig. 7C).

GAs are committed to the developmental events leading to reproductive competence (Cheng *et al.*, 2004; Dill *et al.*, 2004; Achard *et al.*, 2007; Ariizumi *et al.*, 2011; Davière and Achard, 2013). The *sly1.10* mutant showed extensive

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deficiencies in all aspects of reproductive growth, a pattern that has not been altered in SLY1$_{\Delta F}$/s and SLY1$_{\Delta C}$/s plants (Table 2). Overexpression of *PslSLY1* and *PslSLY1*$_{\Delta N}$ recovered reproductive growth to levels comparable with WT plants. Relative to the *sly1.10* mutant control, SLY1/s and SLY1ΔN/s plants exhibited a visible increase in the number of flowers per inflorescence, estimated to be ~3-fold (Table 2). Moreover, SLY1/s and SLY1$_{\Delta N}$/s plants began flowering ~18.5 d earlier than *sly1.10* plants, with no apparent difference from WT plants. Although the *sly1.10* mutant displayed generally smaller flowers, it shared a crucial character with the WT by securing a coordinated flower structure, in terms of a similar pistil/filament length ratio that ensures correct fertilization and seed set. SLY1/s and SLY1$_{\Delta N}$/s plants set larger flowers with accelerated stamen and pistil growth that maintained the proper floral patterning (Table 2; Supplementary Fig. S4). The improvement in various flowering characteristics in SLY1/s and SLY1$_{\Delta N}$/s resulted in an enhancement of reproductive traits, relative to the *sly1.10* mutant (Table 2; Supplementary Fig. S4). In SLY1/s and SLY1$_{\Delta N}$/s plants, the conclusions to silique emergence as well as silique maturation were similar to those of the WT and noticeably shorter than those of the control *sly1.10*. Despite the appropriate flower structure of the *sly1.10* mutant, it showed a notable reduction in seed number. The overall reproductive growth deficiency suggested a strong involvement of compromised GA signaling (Dill *et al.*, 2004; Ariizumi *et al.*, 2008). All disorders in silique length and fertility observed in *sly1.10* mutant plants were considerably recovered to WT levels in SLY1/s and SLY1$_{\Delta N}$/s plants. It has been reported that GA responsiveness is perturbed in *sly1.10* mutant, as the GA applications do not result in changes in growth habit (McGinnis *et al.*, 2003; Dill *et al.*, 2004). Increasing the levels of GA receptors (*GID1* overexpression) or restoring GA signaling machinery (*SLY1* overexpression) in *sly1.10* plants re-establishes GA responsiveness and triggers plant growth via activating the suppression of DELLA repressors (Yasumura *et al.*, 2007; Ariizumi *et al.*, 2008).

PslSLY1 is an active F-box protein involved in GA signaling in plum that can efficiently interact with DELLA proteins *in vitro*. As in the *Arabidopsis* GA signaling model, the formation of the co-receptor GA–PslGID1–PslDELLA complex allows SCF^{PslSLY1} to bind to and ubiquitinate PslDELLA proteins (Hirano *et al.*, 2008, Ariizumi *et al.*, 2011). This model predicts that SLY1 should form a complex that includes not only DELLA but also GID1 proteins. The results reported here present additional confirmation of the indispensable role of GA in organizing fruit development. They provide a foundation for advancing research into the precise nature of the complex development of fruit.

Supplementary data

The following supplementary data are available at *JXB* online.

Table S1. The oligonucleotide primers used for the study.

Table S2. *PslSLY1* promoter analysis using the PLACE website.

Fig. S1. Evolutionary relationships in the GA signal transduction factor SLY1/GID2 and SNE gene family.

Fig. S2. *PslSLY1* transcript regulation in response to the application of GA or the GA biosynthesis inhibitor PAC.

Fig. S3. Effect of overexpressing the full-length *PslSLY1* and truncated derivatives in the rescue of germination defects of the *sly1.10* mutant.

Fig. S4. Effect of overexpressing the full-length *PslSLY1* and truncated derivatives on recovering floral organ and silique growth of the *sly1.10* mutant.

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Author contributions

IE designed the experiments, plant transformation, phenotypical, and molecular characterization of transformed *Arabidopsis*, data analysis, supervised the study, generated the original version of the manuscript, and produced the final version of the manuscript. AI designed the experiment, protein–protein interaction assay, generated Y2H and BiFC constructs, and reviewed and edited the original version of the manuscript. AD performed subcellular localization, Y2H, and BiFC experiments. WE performed plant transformation, and phenotypical and molecular characterization of transformed *Arabidopsis*, and associated data analysis. SJ designed the experiment, provided plum materials, RNA/DNA and qPCR assay, and reviewed and edited the original and final version of the manuscript. SS designed the experiment, and reviewed and edited the original version of the manuscript.

Data availability

All data supporting the findings of this study are available within the paper and within its supplementary data published online.

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