Research review

Plant grafting: how genetic exchange promotes vascular reconnection

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Summary

Grafting has been widely used to improve horticultural traits. It has also served increasingly as a tool to investigate the long-distance transport of molecules that is an essential part for key biological processes. Many studies have revealed the molecular mechanisms of graft-induced phenotypic variation in anatomy, morphology and production. Here, we review the phenomena and their underlying mechanisms by which macromolecules, including RNA, protein, and even DNA, are transported between scions and rootstocks via vascular tissues. We further propose a conceptual framework that characterizes and quantifies the driving mechanisms of scion–rootstock interactions toward vascular reconnection and regeneration.

Introduction

Plant grafting is a vegetative propagation technique that connects two severed plant segments together. The chimera, consisting of the scion and rootstock, survives as a new individual after wound healing. Natural grafting, which occurs when stems or roots of plants attach and fuse (Mudge *et al.*, 2009), has facilitated the invention of classic grafting techniques (Fig. 1). In recent years, micrografting protocols have been used increasingly as a tool to evaluate signaling and transport (Turnbull *et al.*, 2002; Turnbull, 2010).

The success of the graft depends on the compatibility between the rootstock and scion. Studies have indicated that grafts in different genera of the same family are rarely compatible, but grafts of different species within the same genus can survive by forming an effective graft union (Goldschmidt, 2014). The majority of homografts are compatible, with the exception of monocots. Since the wound required for grafting disrupts the plant vascular system (Asahina & Satoh, 2015), reconnection of the vasculature is necessary to maintain normal water and nutrient transportation. Most monocots do not have vascular cambia, which may be a reason why grafting fails (Sachs, 1981; Melnyk & Meyerowitz, 2015). This further suggests that vascular differentiation during wound healing is a prerequisite for successful grafting.

When the cambium of the scion joins fully with that of the rootstock, intact cells divide and proliferate into calli, which eventually differentiate into vasculature and plasmodesmata forms (Melnyk & Meyerowitz, 2015). Although the detailed molecular mechanisms underlying this process require further research, some studies have found that hormones, such as auxin, cytokinin and GA, play a pivotal role in regulating stock–scion interactions (Aloni *et al.*, 2010). Histological and microarray analyses of *Arabidopsis* micrografting identified auxin, ethylene and jasmonic acid as important molecules that participate in development of the graft union, and a model has been proposed to better interpret this phenomenon (Yin *et al.*, 2012; Fig. 2).

After cell walls fuse in the graft union, plasmodesmata stretch in small groups over the spaces of the inner cell wall, interconnecting the protoplasts of contiguous cells (Kollmann & Glockmann, 1985). Heterogeneous cells then interdigitate through the plasmodesmata (Melnyk & Meyerowitz, 2015). The plasmodesmata provide tunnels for small molecules and even selectively permit the movement of macromolecules, such as proteins and nucleic acids. Additionally, vascular reconstruction at the graft union enables macromolecules to be transported (Harada, 2010). In recent years, increasing effort has been made to determine how macromolecules are transferred between scions and rootstocks in grafting plants to reveal the mechanisms that control graft-induced changes in plant traits (Paultre *et al.*, 2016).

In this review, we first describe several different types of graft-induced phenotypic changes. We highlight existing evidence for the molecular and physiological mechanisms underlying grafting and then propose a framework to interpret how the transportation of genetic materials between the scion and rootstock is related to vascular reconnection and regeneration.

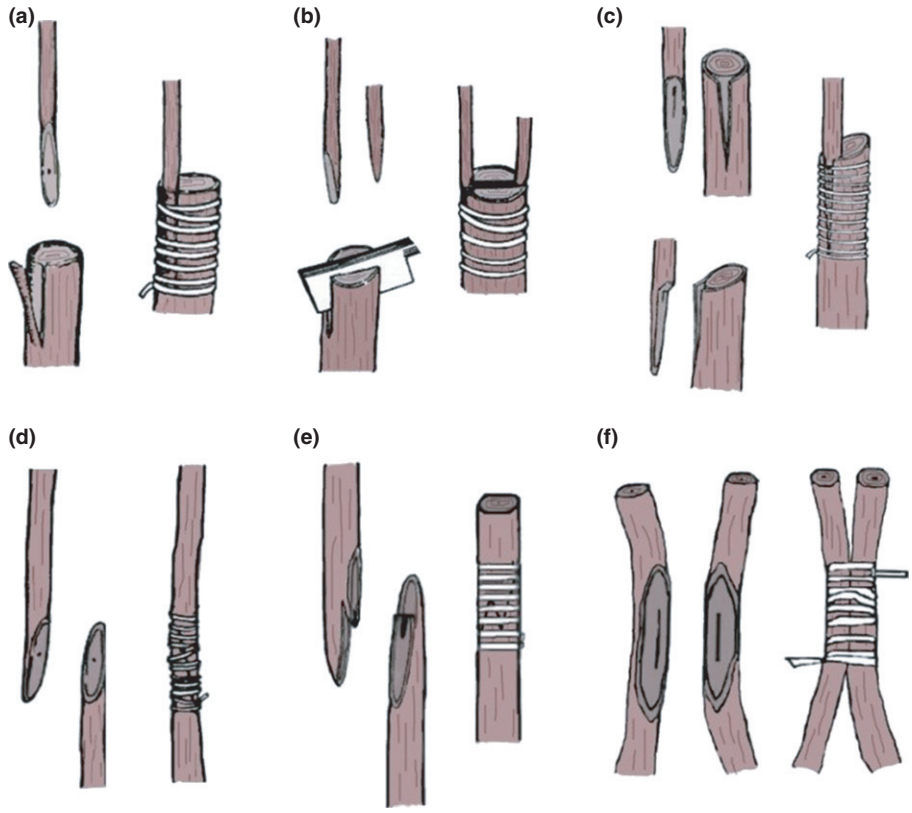

Fig. 1 Different types of traditional grafting methods. (a) Cut grafting, (b) cleft grafting, (c) crown grafting, (d), splice grafting, (e) tongue grafting, (f) approach grafting.

Phenotypic variation in grafted plants

Grafting commonly influences the phenotype of the grafted plants (Warschefsky *et al.*, 2015), including changes in fruit quality, resistance to pests and pathogens, tolerance to adversity and stress, and other physiological disorders. The vegetative fruit quality of scions is commonly altered by the rootstocks after grafting. Taller *et al.* (1998) described a case in which two pepper scion cultivars acquired changes in fruit shape, color and pungency after grafting. The results also illustrated that several rootstock features were present in the progeny of the scion after self-pollination. Similarly, a graft of three watermelon cultivars and three hybrid squashes showed differences in shape, weight, yield, quality, rind thickness and pH among stocks (Turhan *et al.*, 2012). Fruit trees, such as sweet cherry, apple and citrus, have also been shown to be influenced by grafting.

Grafting is widely used to improve resistance to pests and diseases. For instance, grafting can alleviate the development of post-harvest diseases in Hass avocado fruit (Willingham *et al.*, 2001). Anthracnose, caused by the fungus *Colletotrichum gloeosporioides*, is the most severe post-harvest disease of avocado fruits. The rootstocks can significantly affect the post-harvest anthracnose resistance of scions, which is probably related to an increase in antifungal diene and improvement in mineral nutrients in the scions. Research on the resistance of pepper plants to both phytophthora blight and bacterial wilt also confirmed the effect of grafting. Five commercial stocks and nine breeding lines were used as rootstocks for the scion 'Nokkwang', three of which were selected for their greater resistance to phytophthora blight and bacterial wilt without reduction in productivity or fruit quality (Jang *et al.*, 2012). Furthermore, a study using tomatoes revealed that cultivars grafted onto nematode-resistant rootstocks gained higher yields than did nongrafted ones (Lopez-Perez *et al.*, 2006). Similar results have been found in eggplants (Ioannou, 2001), cucumbers (Gu *et al.*, 2006) and peppers (Oka *et al.*, 2004).

Grafting can also affect tolerance to abiotic stress. Experiments in cherry tomato found that grafting on drought-tolerant rootstocks resulted in higher fruit production (Sánchez-Rodríguez *et al.*, 2012). Several studies have shown that salt stress in cucumber can be alleviated by grafting cucumber onto *Cucurbita* rootstocks. Alleviation of salt stress may be due to delaying photo-inhibition, which is caused by changes in nitrogen metabolism during salt penetration. Other examples, such as the tolerance of citrus to boron stress (Papadakis *et al.*, 2004a,b) and the resistance of tomato to thermal stress (Rivero *et al.*, 2003), provide more options for exploring the mechanisms of this phenomenon.

In addition to the cases mentioned above, related publications have indicated that physiological and morphological features can be altered by stock–scion interactions. The ability of rootstocks from certain fruit trees to dwarf their scions, which has been acknowledged for decades, is used in agriculture. A series of rootstocks used for dwarfing has been developed in apples, and genetic marker analysis linked to the dwarfing traits has been performed. In micrografting experiments in *Arabidopsis*, Turnbull *et al.* (2002)

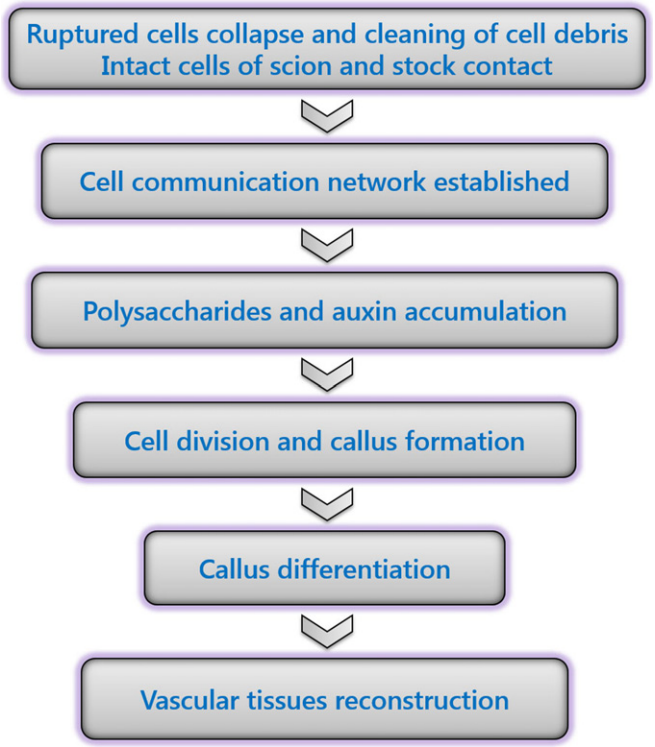

Fig. 2 A concise model for anatomical changes occurring in graft-union formation. The chart describes the wound healing process at the graft junction, which can be used for studies of graft-union formation based on both micrografting and traditional grafting techniques. This model illustrates the stages of signal exchange, especially hormone exchange, which play pivotal roles in subsequent processes of grafting (Yin *et al.*, 2012).

found that the wild-type (WT) stocks can effectively inhibit rosette branching of the increased branching mutants *max*1 (more axillary growth) and *max*3. Notably, when two shoots from the *max*1 and WT seedlings were simultaneously grafted onto a *max*1 rootstock, the mutant shoot showed increasing branching while the WT shoot did not. When the *max*1 rootstock was replaced with a WT rootstock, neither of the shoots branched profusely. The results indicated that branch signaling can spread from root to shoot but not from shoot to shoot.

The majority of current research has been dedicated to using rootstocks to influence shoot phenotypes, but the root changes induced by scions have been seldom discussed, probably due to the important role that scions play in agricultural and horticultural practices and also the relative difficulty in observing root phenotype changes given that they are below ground. However, the effects of the scion on stock growth and carbohydrate storage seem to be indisputable (Dahniya *et al.*, 1982), and root–shoot interactions remain to be explored further.

Molecular interactions between scions and rootstocks

Graft-induced phenotypic changes have triggered research into the endogenous factors that control rootstock–scion interactions. Historically, botanists believed that plant hormones are responsible for these interactions due to their roles in regulating plant vegetative growth and reproduction. Hormones in plants are regulated by feedback loops and remain balanced in nongraft plants. A study of peach grafts demonstrated that hormonal balance is disrupted after grafting (Sorce *et al.*, 2002). Since many plant hormones are highly mobile, they can be translocated easily in the graft chimeras. According to existing evidence, hormonal signaling is involved in root–shoot interactions, including graft-union formation, scion–rootstock communication, and plant growth and development (Aloni *et al.*, 2010).

Horizontal gene transfer

The heritability of graft-induced phenotypic changes suggests that regulatory processes underlying the scion–rootstock communication also involve a genetic component (Taller *et al.*, 1998; Tsaballa *et al.*, 2013). In fact, the presence of heritability coincides with Lysenko's graft hybrid hypothesis, which suggests that graft hybridization has similar properties to those of sexual hybridization. This concept, which seems to be inconsistent with Mendelian genetics, was initially rejected by Western scientists, but research over recent decades has provided evidence for the existence of graft hybridization. Pandey (1976) proposed that fragments of chromatin produced by cells rupture after grafting and then can migrate into their neighboring cells via plasmodesmata. Later, Ohta (1991) provided evidence that chromatin can move via the vascular bundles from the lignifying stock, across the graft union, and into the growing point of the scion. In addition, a study by Taller *et al.* (1998) detected several random amplification of polymorphic DNA (RAPD) markers in the graft-induced variants and found the same bands in the rootstock cultivar but not in the scion. They suggested that the genetic changes caused by grafting were attributable to direct DNA uptake through the vascular bundles.

To demonstrate exchange of genetic materials between cells in grafted plants, Stegemann & Bock (2009) created two transgenic tobacco lines that harbor different marker and reporter genes in their nuclear and chloroplast genomes, respectively. These two lines were reciprocally grafted, with the grafted stem regions exposed to resistance selection. In subsequent reporter expression experiments, plastid genes were found to transfer short distances across the graft union, indicating an opportunity for grafting to pursue horizontal gene transfer (HGT). HGT is the asexual transfer of genetic materials from a donor organism to a recipient organism, playing an important part in eukaryotic genome evolution (Bock, 2010). Plasmodesmata formation and re-establishment of vascular bundles provide transport channels for HGT during formation of the graft union. To investigate whether large DNA fragments or whole organelles are involved in gene transfer, Stegemann *et al.* (2012) generated three other tobacco lines (transplastomic *Nicotiana tabacum*, transgenic *N. glauca* and *N. benthamiana*) for graft experiments. After sequencing two polymorphic regions distant from the transgenic regions on the plastid differing substantially among the three lines, identical sequences from *N. tabacum* in *N. tabacum*/*N. glauca* and *N. tabacum*/*N. benthamiana* grafts were obtained. Since there is no genome recombination between scions and stocks, this finding indicates

that the entire plastid genome travels through the graft union (Stegemann *et al.*, 2012).

Despite horizontal transfer of DNA was confirmed to occur across the graft site, the authors stated that 'they do not lend support to the tenet of Lysenkoism that "graft hybridization" would be analogous to sexual hybridization' because of the restriction of gene transfer to short distances close to the graft site. Nevertheless, they offered a bolder suggestion that grafting could be an asexual path to speciation. In the experiments of grafting *Nicotiana* plants in which the graft unions were maintained in tissue culture after resistance selection, Fuentes *et al.* (2014) found that nuclear genome transfer between scion and stock has occurred, producing new fertile and stable allopolyploid species at a considerable rate. Thus, grafting could lead to a direct transfer of the entire nuclear and plastid genomes across the graft junction, which has widespread implications for understanding grafting mechanisms and plant evolution.

Epigenetic and mobile small RNAs

Epigenetic modifications may be other potential roles in creating heritable phenotypic variation via grafting. Epigenetics is the study of heritable variations in gene expression that are not caused by differences in DNA sequence as a result of modifications, such as DNA methylation or structural changes to chromatin. A study in interspecies Solanaceae grafting showed that locus-specific alterations in DNA methylation were produced in the grafting process and that these alterations in the grafted scions are partially heritable to their self-pollinated progenies (Wu *et al.*, 2013). Moreover, small RNA (sRNA)-mediated graft-transmissible epigenetic modifications have been detected in *Arabidopsis thaliana* grafting experiments. Molnar *et al.* (2010) showed that 24 nt sRNAs that have transferred from shoots to roots can cause epigenetic changes by mediating DNA methylation at three sites in the rootstock cells. A follow-up study showed that the mobile sRNAs acted on RNA-directed DNA methylation at thousands of loci genome wide. A small number of genes characterized by transcriptomic analysis in recipient tissues were correlated with mobile small interfering RNAs (siRNAs) and DNA methylation (Lewsey *et al.*, 2016).

The 24 nt sRNAs, as well as 21–23 nt sRNAs, are key components in gene silencing. These sRNAs are produced by the activities of DICER-LIKE proteins and then loaded onto ARGONAUTE proteins (AGOs) to target RNAs (Borges & Martienssen, 2015; Table 1). RNA gene silencing signals are distributed systemically in plants and are capable of transmitting across the graft union in grafting plants (Chitwood & Timmermans, 2010; Fragoso *et al.*, 2011). All classes of both transgene-specific siRNAs and endogenous siRNAs showed mobility between graft partners in *A. thaliana* grafting studies (Molnar *et al.*, 2010). The mobile siRNAs from the rootstock were reported to induce endogenous post-transcriptional gene silencing in the scion, and 24 nt siRNAs from the shoots have also been found to direct transcriptional gene silencing in the rootstock cells (Melnyk *et al.*, 2011). In another report, Dunoyer *et al.* (2010) provided evidence that 21 nt siRNA duplexes function as mobile silencing signals among cells.

Table 1 sRNA silencing pathways in plants

sRNA class	sRNA size (nt)	DICER-LIKE proteins	Associated AGO proteins	Functions
miRNA	21	DCL1	AGO1	Target transcript cleavage Translation inhibition
trans-acting siRNA	21	DCL4	AGO1 AGO7	Target transcript cleavage
Viral siRNA	21 22	DCL4 DCL2	AGO1 AGO2	Target transcript cleavage
Heterochromatic siRNA	23 24	DCL3	AGO4 AGO6 AGO9	Chromatin modification DNA methylation

Several miRNAs have also been shown to be graft-transmissible signals that target a wide range of transcripts. Phosphate starvation-induced miR399 increased in concentration under phosphate limitation and functioned as a long-distance signal, exerting its biological function in the recipient cells (Pant *et al.*, 2008). miR395 was also transported across the graft site and down-regulated one of its targets during nutrient starvation (Buhtz *et al.*, 2010). miR156 and miR172, which regulate the transition from juvenile to adult development in plants, were reduced in expression after grafting and led to graft-transmissible induction of tuber yields (Martin *et al.*, 2009; Bhogale *et al.*, 2014). In summary, changes in sRNA abundance after grafting are important factors for initiating graft-induced changes in gene expression.

Transmissible messenger RNAs and proteins

Protein-coding messenger RNAs (mRNAs) might also be important signaling molecules involved in plant grafting. Transported mRNAs may act as regulatory signals or produce functional proteins in the targeted organs. Haywood *et al.* (2005) used grafting experiments to show that long-distance transport of *gibberellic acid insensitive* (*GAI*) RNA via the phloem alters leaf morphology. A further study indicated that *GAI* RNA contains specific elements for long-distance transport, as the fused transcript of *GAI*-encoding sequence and *GREEN FLUORESCENT PROTEIN* (*GFP*) RNA could move a long distance, but free *GFP* could not. Analysis on the *GAI* sequence motif revealed that coding sequences and 3′ untranslated region (UTR) are necessary for the movement. In addition, distinct tertiary structure may be the RNA movement machinery, because recovery of the secondary structure in movement-defective *GAI* RNA cannot result in the entire rescue of RNA movement (Huang & Yu, 2009). Coincidentally, the 3′ UTRs of the phloem-mobile transcript *StBEL5* in potato also proved to play important roles in mRNA stability and trafficking into roots (Banerjee *et al.*, 2009; Cho *et al.*, 2015). To further explore the RNA motifs triggering mobility and the fate of transported mRNAs in target tissues, a recent study by Zhang *et al.* (2016) produced mRNAs harboring distinctive tRNA-like structures (TLSs), and found that these mRNAs could move across chimeric

graft junctions and could be translated into proteins after transport. The results suggest that tRNA-derived sequences with a motif are necessary to trigger mRNA transport to distant plant cells (Zhang *et al.*, 2016), providing a potential explanation for the molecular mechanisms enabling intercellular mRNA transport and the fate of transported mRNAs.

Using high-throughput RNA sequencing, recent work on interaccession grafting in *Arabidopsis* indicated that many transcripts were bidirectionally mobile across the graft site through the vasculature. Proteomic analysis in graft tissues raised the possibility that mRNAs may be translated after transfer and may have a broad range of functions (Thieme *et al.*, 2015). Large-scale transcript exchange among species has also been verified by sequencing in *Cuscuta pentagona* and its hosts (Kim *et al.*, 2014). This transcript exchange is likely to be similar to plant grafting, as the haustoria of *Cuscuta* can penetrate host tissues and form vascular connections. These large-scale movements of mRNA show similarities to HGT, and we can then speculate that they play pivotal roles in recipient tissues. Dealing with the large-scale high-throughput sequencing data, Calderwood *et al.* (2016) proposed a default pathway to reveal the distribution of the mobile and nonmobile mRNA species. They suggested that nonsequence-specific movement of mRNA in the phloem may be directly related to transcript abundance and half-life within companion cells. This model offers an available path to explain large-scale mobile transcripts (Calderwood *et al.*, 2016).

It has been verified that some proteins are capable of binding mRNAs as chaperones during transport from source to sink tissues. These RNA-binding proteins can facilitate transport and protect mRNAs from degradation. CmPP16, which was detected in the phloem of *Cucurbita maxima*, was the first chaperone reported. Using grafting experiments, CmPP16 was shown to be transported with the mRNA from rootstock to scion (Xoconostle-Cázares *et al.*, 1999). In a recent study, PbPTB3, a member of the PTB family of proteins in the pear variety 'Du Li' (*Pyrus betulaefolia*) that binds to numerous mRNAs, was shown to be transported long distances in the phloem and to bind the CUCU domain of *PbWoxT1* mRNA (Duan *et al.*, 2015a,b). The binding of PbPTB3 to *PbWoxT1* mRNA promotes its long-distance transport across the graft union. Several other proteins can also move long distances between tissues and act as regulators. For instance, a phloem-mobile cyclophilin in tomato, SICyp1, was found to move from scion to rootstock. This long-distance transport was associated with an increased auxin response, leading to enhanced root growth (Spiegelman *et al.*, 2015). In a recent study, Paultre *et al.* (2016) used a grafting system to address protein trafficking, and found extensive movements of proteins from the shoot companion to the root stele cells. Similar to mRNAs, the abundance of proteins might affect protein trafficking. These studies substantially support the occurrence of a long-distance transport of mRNAs and proteins, which can even fulfill their functions and alter plant morphological development (Table 2). The most recent reports mentioned above provide valuable guidance for new studies to explain how mRNAs and proteins are triggered to transport and whether their mobility occurs specifically or as a result of their abundance in the phloem companion cells.

Molecular mechanisms underlying vascular reconnection

Transportation of molecular signals in grafting plants occurs mainly in the phloem, which requires to the vascular tissues to be reconnected after wound healing. The reconnection process has been well characterized, but the molecular mechanisms underlying this process remain poorly understood. It has been confirmed, however, that plant hormones are involved in vascular reconnection. Auxin accumulation can promote the differentiation of callus cells into xylem and phloem (Wetmore & Rier, 1963). In *Arabidopsis*, auxin transport plays a pivotal role in the promotion of wound healing and leaf vascular formation and development. Auxin transport acts in feedback loops and is modulated by many auxin response factors. Cytokinin is another critical regulatory hormone involved in wound response due to the importance of

Table 2 mRNAs/proteins capable of long-distance transport and their potential function in grafting plants

mRNAs	Proteins	Target traits	Species	Reference(s)
CmNACP		Meristem development	Pumpkin	Ruiz-Medrano *et al.* (1999)
CmPP16	CmPP16		Pumpkin, rice	Xoconostle-Cázares *et al.* (1999), Aoki *et al.* (2005)
Me		Leaf morphology	Tomato	Kim *et al.* (2001)
CmmLec17	CmmLec17		Melon	Gomez *et al.* (2005)
St BEL5	POTH1	Tuber formation	Potato	Banerjee *et al.* (2006, 2009), Cho *et al.* (2015)
PFP-LeT6		Leaf development	Tomato	Kudo & Harada (2007)
GAI		Leaf development	*Arabidopsis*, tobacco, apple	Haywood *et al.* (2005), Huang & Yu (2009), Xu *et al.* (2010, 2013)
FT	FT	Promotion of flowering	*Arabidopsis*, rice, potato, pumpkin	Corbesier *et al.* (2007), Jaeger & Wigge (2007), Lin *et al.* (2007), Tamaki *et al.* (2007), Navarro *et al.* (2011), Lu *et al.* (2012)
Aux/IAA		Root development	*Arabidopsis*, melon	Notaguchi *et al.* (2012), Golan *et al.* (2013)
RBP50	RBP50		Pumpkin	Ham *et al.* (2009), Li *et al.* (2011)
AtTCTP2	AtTCTP2	Root development	*Arabidopsis*	Toscano-Morales *et al.* (2014)
PbWoxT1	PbPTB3	Growth and flower development	Pear	Duan *et al.* (2015a,b)
	SICyp1	Root development	Tomato	Spiegelman *et al.* (2015)
PBKN1	PbMPB2C	Microtubule accumulation	Pear	Duan *et al.* (2015a,b)

division and differentiation in regeneration. It has been reported that cytokinin is involved in the wound induced differentiation 1 (WIND1) pathway, which controls cell dedifferentiation after wounding. Regulation by cytokinin signaling and its reciprocal interaction with *Arabidopsis* histidine phosphotransfer protein 6 (AHP6) crucially affects cell proliferation and differentiation during vascular development (Mähönen *et al.*, 2006). In fact, auxin and cytokinin are likely to be involved simultaneously in the same vascular development processes and to participate in the other's functional pathways. Beyond these two main phytohormones, other hormones, such as ethylene and jasmonic acids, have been found to play regulatory roles in tissue reconnection (Asahina *et al.*, 2011; Yin *et al.*, 2012).

Recently, to further investigate how grafts reconnect, Melnyk *et al.* (2015) conducted a series of elegant experiments in *Arabidopsis* and revealed several temporal events during graft formation. They pinpointed the precise timing of vascular tissue attachment using fluorescence observations and determined the following order of events: phloem reunion, root growth and xylem reunion. Next, they examined cell division and differentiation at the graft site, finding that rootstock and scion contribute asymmetrically to graft formation and both phloem and xylem reconnections are promoted by an apically derived signal. The roles of auxin and cytokinin responses in vascular reconnection were subsequently investigated by monitoring reporters of these two hormones at the graft union and analyzing the rates of phloem connection in mutants that lack auxin or cytokinin responses. The results indicated that a subset of auxin-affected mutant rootstocks significantly delay phloem connection, indicating that auxin is an essential factor for vascular reconnection. Among these mutants, ALF4 and AXR1 were suggested to be required below the graft site and to contribute to phloem reunion, and ALF4 is further required to initiate an auxin response in the rootstock. Altogether, Melnyk *et al.* (2015) demonstrated complex communication involved in graft junction reconnection. They revealed potential signals and auxin-related genes that regulate graft formation and vascular reconnection.

In addition to the genes involved in hormone pathways, other factors related to vascular development may also aid in graft formation. For instance, two factors, ALTERED PHLOEM DEVELOPMENT (*APL*) and OCTOPUS (*OPS*), are reportedly involved in the regulation of phloem differentiation. *APL* is a gene encoding an MYB coiled-coil-type transcription factor that is necessary for plant specification in *Arabidopsis*. Studies by Bonke *et al.* (2003) showed that *APL* is both a promoter of phloem differentiation and an inhibitor of xylem differentiation. *OPS* is another gene identified to be an inducer of phloem differentiation and essential for phloem continuity (Truernit *et al.*, 2012). A polarly localized membrane-associated protein encoded by *OPS* was reported to have an antagonistic interaction with BIN2 in the regulation of phloem differentiation (Anne *et al.*, 2015). The class III homeodomain-leucine zipper (HD-ZIP) gene family, which comprises Revoluta, Phabulosa, Phavoluta, ATHB8 and Corona/ATHB15, has been shown to regulate vascular development (Prigge *et al.*, 2005). Members of this family were found to promote xylem differentiation. In roots, class III HD-ZIP members are regulated by an RNA interference mechanism via miR165/166. The mobile miR165/166 in the root is critical for regulating its target mRNAs that encode class III HD-ZIP transcription factors in xylem differentiation (Miyashima *et al.*, 2011). Identifying additional regulatory pathways and modifications of transcription factors during vascular reconnection may be challenging but will help us to better understand the mysteries of grafting.

A mechanistic framework to model scion–rootstock interactions

A successful graft is based on vascular reconnection. The possible molecular mechanisms that lead to vascular reconnection have been discussed in this article. Most of the evidence demonstrates that molecular signaling plays an important role in the process of graft formation (Miyashima *et al.*, 2011; Melnyk *et al.*, 2015). It is possible that graft compatibility is associated with the molecular signaling discussed above, and sharing genes or mobile factors may be essential to regulate vascular reconnection. We propose a conceptual framework to model and analyze how these regulators interact and coordinate to reconnect and regenerate vasculature and plasmodesmata after grafting.

Suppose that we initiate a grafting experiment in which scions from two sharply contrasting genotypes A and B in shoot architecture and rooting capacity are self-grafted onto their own rootstocks, respectively, and in the meantime, these two genotypes are reciprocally grafted, leading to four grafting combinations A × A, A × B, B × B and B × B (Fig. 3). In each combination, gene expression on one tissue may be activated or repressed by gene expression on another tissue and, as evidenced from previous grafting experiments, such regulatory circuits participate in

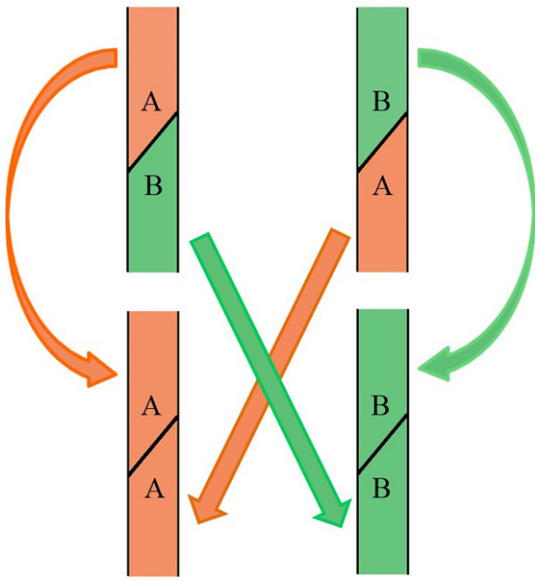

Fig. 3 A grafting experiment, including two self-grafts and two reciprocal grafts between two hypothetical sharply contrasting genotypes A (orange) and B (green), designed to build a modeling framework for the molecular mechanisms of scion–rootstock interactions.

vascular reconnection and regeneration (Stegemann & Bock, 2009; Melnyk *et al.*, 2015).

By treating the scion and rootstock of a grafting combination as a system, we incorporate two coupled ordinary differential equations (ODEs) to characterize dynamic interactions of gene expression derived from the two tissues and interpret these interactions according to game theory. Each equation describes the change rate of gene expression on a different tissue, which dissolves the expression of the target tissue into two parts, the independent expression and dependent expression. The independent part describes the amount of gene expression that takes place by assuming that the target tissue is not grafted, whereas the dependent part represents the amount of gene expression that arises from the interaction of the target tissue with its grafting counterpart through some mechanisms. By estimating ODE parameters that define the sign and magnitude of the second part, scion–rootstock interactions can be quantified and further interpreted by game theory. Game theory, originated from von Neumann & Morgenstern's (1944) economic work in the early 20th century and recently used to map the genetic architecture of complex traits (Zhu *et al.*, 2016), was developed to study the conflict and cooperation of different players in a system. Each player tends to make an optimal strategic decision to acquire maximum payoffs in interactions. With the ODE parameters estimated from each grafting combination, this theory can be renovated to interpret the way the scion interacts with the rootstock to obtain the optimal fitness of each tissue.

We further build a unifying framework that can compare the pattern of scion–rootstock interaction and communication, determined by gene expression, among different grafting combinations. Previous studies have shown that graft-induced phenotypic changes may be due to the exchange of genetic materials between different tissues (Taller *et al.*, 1998; Stegemann & Bock, 2009; Tsaballa *et al.*, 2013; Melnyk *et al.*, 2015). Because of this, grafting has been thought to be an evolutionary driving force for plant speciation

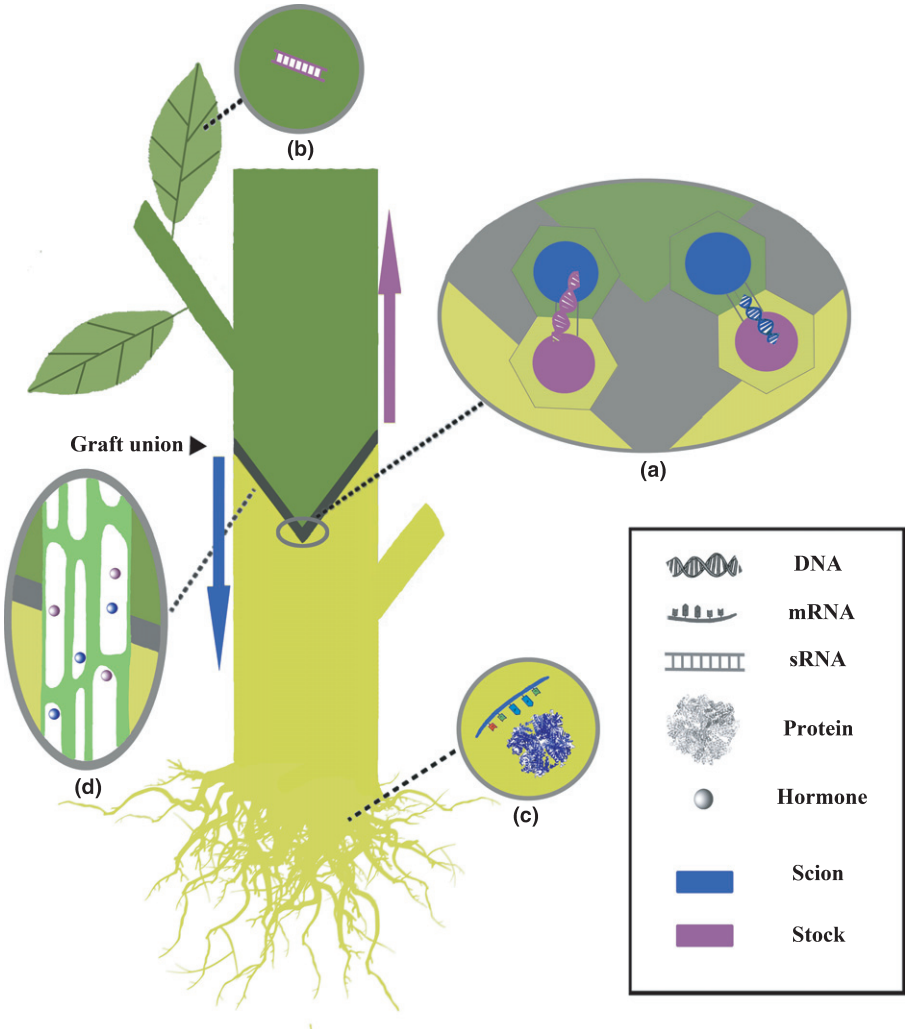

Fig. 4 Schematic view of molecular signals transported between scion and rootstock in grafting plants. (a) Horizontal transfer of DNA via plasmodesmata at the graft site. (b) sRNAs move across the graft union to distant tissues and function as silencing signals. (c) Long-distance trafficking of mRNA molecules. (d) Hormonal signals play important roles in scion–rootstock interactions. Different colors represent molecules from different tissues. Blue represents molecules from scion, and purple represents molecules from rootstock. The triangle indicates the graft union of the grafting plants. The arrow indicates that the molecules can be transported bidirectionally.

(Fuentes *et al.*, 2014; Goldschmidt, 2014). By comparing the difference of scion–rootstock interactions between combinations A × A and A × B as well as combinations B × B and B × A, the unifying framework helps us to understand how the rootstock drives the evolution of the shoot form through genes or other regulators identified. Reciprocally, the comparison of combinations A × A and B × A as well as combinations B × B and A × B can shed light on the evolution of the rooting system driven by the scion.

Conclusions and future prospects

Grafting is an easy and rapid vegetative propagation technique that can overcome the problem of sexual incompatibility between two different species. According to Fuentes *et al.*'s (2014) research, entire nuclear genomes can transfer between plant cells of scion and stock, leading to the formation of new alloploid derived different species. Studies on the molecular mechanisms behind these phenotypic changes may provide insight into optimal breeding methods (Goldschmidt, 2014; Melnyk & Meyerowitz, 2015). The interactions between scions and rootstocks are very complex, but increasing research has attempted to uncover the processes involved in these interactions and the resulting graft-induced phenotypic changes.

Endogenous factors have been found to move across the graft union, which is the most convincing explanation for how phenotypic changes are induced in grafts. In addition, HGT between scion and rootstock and siRNA-mediated graft-transmissible epigenetic modifications may provide a possible explanation for heritable variations after grafting, while rigorous evidence is desperately awaited. In the current study, DNA transfer was confined to the graft union and did not spread long distances (Stegemann & Bock, 2009) (Fig. 4a). The confined movement of DNA may not sufficiently explain how particular trait variation occurs, so the possibility of long-distance transfer of DNA should be addressed in future research. Evidence has also been presented that nuclear HGT occurred in parasitic plants (Yoshida *et al.*, 2010). Using phylogenetic analysis, the authors concluded that the eudicot parasite requires a nuclear gene from its monocot hosts, indicating the significance of HGT in nature. Endogenous mobile siRNAs in grafting plants, by contrast, function as silencing signals directing epigenetic modifications and transcriptional gene silencing in the recipient tissues, which may play roles in the adaption of grafted plants to external stimuli (Fig. 4b). Heterografting experiments also provided evidence for long-distance transcript or RNA–protein complex trafficking, which plays significant regulatory roles in the response to developmental processes (Fig. 4c). Phytohormones have also been found to be crucial for plant growth. With the exception of straightforward translocation of hormones during scion–stock interactions, the regulation of hormone levels, through the trafficking events mentioned above, is probably complex (Fig. 4d). The finding that movement of particular factors has been detected in different grafting species, such as the FT protein found in *Arabidopsis*, rice and pumpkin (Lin *et al.*, 2007), suggests the universality of such transport.

To date, numerous questions still need to be addressed. Recent works have shed light on what triggers movement of the molecules (Paultre *et al.*, 2016; Zhang *et al.*, 2016), but the more detailed mechanisms enabling intercellular molecular transport still need further research. Extensive macromolecules, including DNAs, RNAs and proteins, have been proved to move between the scion and stock, but a question naturally arises about the way these molecules interact with each other. Does any other undiscovered pathway exist to effect graft-induced phenotypic changes? Furthermore, transportation of cellular signals is a dynamic process, so it will be important to monitor the transport of molecules at different post-graft time points. We have proposed a framework by unifying differential equations and these dynamic data through game theory to identify those genes and regulators that are involved in vascular formation.

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