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REVIEW

Control of grain size in rice

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Key message **Summary of rice grain size**.

Abstract Rice is one of the most important crops in the world. Increasing rice yield has been an urgent need to support the rapid growth of global population. The size of grains is one of major components determining rice yield; thus, grain size has been an essential target during rice breeding. Understanding the genetic and molecular mechanisms of grain size control can provide new strategies for yield improvement in rice. In general, the final size of rice grains is coordinately controlled by cell proliferation and cell expansion in the spikelet hull, which sets the storage capacity of the grain and limits grain filling. Recent studies have identified several quantitative trait loci and a number of genes as key grain size regulators. These regulators are involved in G protein signaling, the mitogen-activated protein kinase signaling pathway, the ubiquitin–proteasome pathway, phytohormone signalings, or transcriptional regulation. In this review, we summarize current knowledge on grain size control in rice and discuss the genetic and molecular mechanisms of these grain size regulators.

Keywords Rice · Grain size · Grain length · Grain width · Grain yield

Rice is one of the most important cereal crops in the world and is also the primary food source for about half of the world's population. Given the rapid increase in global population, improving grain yield has been an urgent need in rice breeding. Rice grain yield is mainly determined by three components: number of panicles per plant, number of grains per panicle, and grain weight. Grain weight is positively associated with grain size. Therefore, grain size is an important agronomical trait for yield improvement in rice.

The rice grain has a typical structure of cereal grains (Fig. 1). The embryo and the endosperm are enclosed by a thin seed coat and covered by the spikelet hull (the husk). The endosperm storing starches and other nutritious compounds occupy the bulk of the mature seed and are the major consumable parts for food. The spikelet hull consists of the palea and the lemma. It not only provides a protective coat, but also forms the container of filling. As the spikelet hull sets the storage capacity of the grain and limits grain growth, it plays a predominant role in determining grain size.

The development of spikelet hull is coordinately regulated by cell proliferation and cell expansion. During early developmental stages, cells in the spikelet hull undergo extensive division to increase cell number. Subsequently, cell division slows down gradually and cell expansion initiates to increase cell size. The final cell number and cell size in different dimensions of the spikelet hull determine grain length, grain width, and grain thickness, therefore influencing the grain size and shape (Fig. 2). Recent studies have identified several quantitative trait loci (QTLs) and a number of genes as key grain size regulators. These regulators have been involved in multiple signaling pathways, including G protein signaling, the mitogen-activated protein kinase (MAPK) signaling pathway, the ubiquitin–proteasome pathway, phytohormone signalings, and transcriptional regulatory factors (Fig. 2, Table 1). In this review, we summarize these findings and discuss the genetic and molecular mechanisms of these regulators in grain size control.

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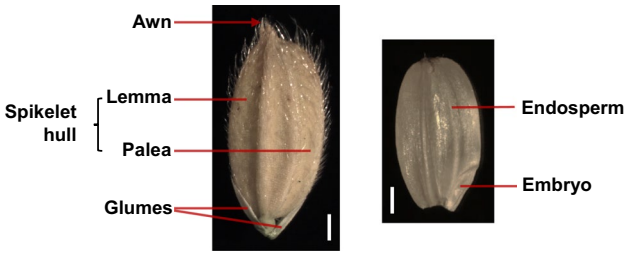

Fig. 1 Overview of rice grains. The left panel shows a mature rice grain. The spikelet hull consists of a palea and a lemma. The right panel shows a brown rice grain. *Bars* 1 mm

Control of grain size by G protein signaling

G protein signaling has been involved in a variety of growth and developmental processes in plants and animals. The heterotrimeric G protein complex consists of three subunits: Gα, Gβ, and Gγ. They function with membrane-bound G protein-coupled receptors (GPCRs) to mediate signal transduction to downstream effectors (Hamm 1998). Recent studies suggest that G protein signaling plays a role in grain size control.

GRAIN SIZE3 (*GS3*) is the first molecularly characterized QTL for grain size. It is the major QTL that contributes to grain-length differences between *indica* varieties and *japonica* varieties. The *GS3* locus was identified by map-base cloning using near-isogenic lines from cross between Minghui 63 (large grain) and Chuan 7 (small grain) (Fan et al. 2006). *GS3* encodes a transmembrane protein with four putative domains: a plant-specific organ size regulation (OSR) domain in the N-terminus, a transmembrane domain, a tumor necrosis factor receptor/nerve growth factor receptor (TNFR/NGFR) family cysteine-rich domain, and a von Willebrand factor type C (VWFC) in the C terminus (Fan et al. 2006; Mao et al. 2010). Intriguingly, the four domains in GS3 protein function differentially in grain size regulation (Mao et al. 2010). The OSR domain is both necessary and sufficient to limit grain size, whereas the C-terminal TNFR/NGFR and VWFC domains have an inhibitory effect on the OSR function. A nonsense mutation carried by the Minghui 63 allele ($gs3^{C165A}$) causes loss of function of the OSR domain, resulting in long grains. A 1-bp deletion carried by Chuan 7 allele ($gs3^{del357}$) results in deletion of most of the C-terminal cysteine-rich region, leading to super short grains. By contrast, Zhenshan 97 harboring the wild-type *GS3* allele produces medium grains. Sequence analysis of 82 accessions revealed that the $gs3^{C165A}$ allele carried

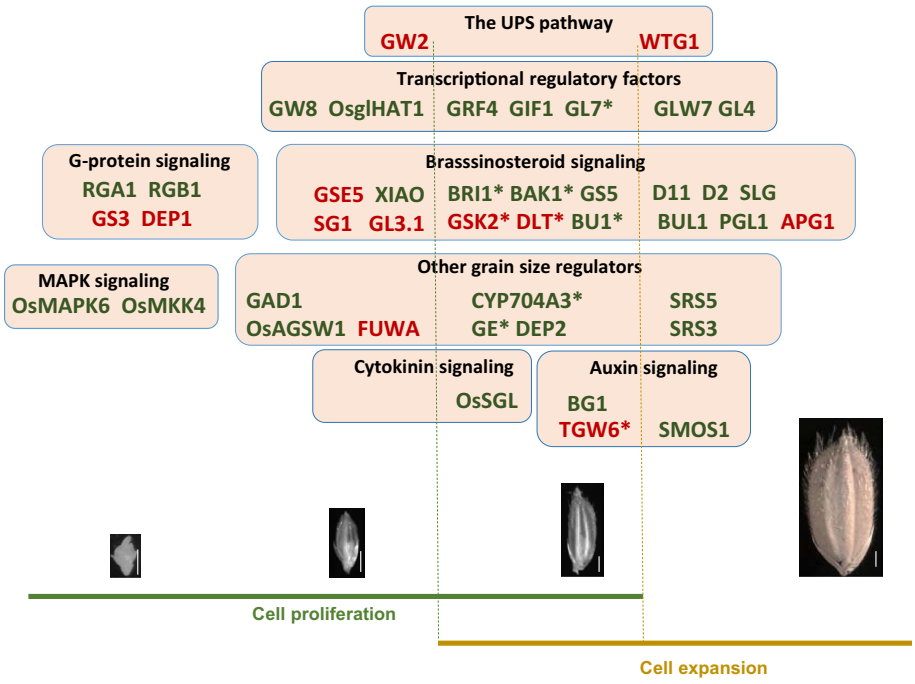

Fig. 2 Grain size control in rice. The grain size of rice is regulated by cell proliferation and cell expansion in the spikelet hull. Components of the ubiquitin–proteasome (UPS) pathway, G-protein signaling, MAPK signaling, phytohormones and transcriptional regulatory factors are involved in grain size control. The positive regulators and negative regulators are shown in green and red, respectively. Regulators controlling cell proliferation are placed to the left of the green dotted line; regulators controlling cell expansion are placed to the right of the yellow dotted line; regulators controlling both processes are placed between the green and the yellow dotted lines. *, insufficient evidence or inconsistent results of their roles in cell proliferation/cell expansion process. *Bars*, 1mm

Table 1 List of factors involved in rice grain size control

Protein name	Accession number	Protein category	Positive (+)/negative (−) regulator	Affecting cell proliferation/expansion	References
The ubiquitin pathway					
GRAIN WIDTH 2 (GW2)	Os02g0244100	E3 ubiquitin ligase	−	Proliferation	Song et al. (2007)
OsOTUB1/WIDE AND THICK GRAIN 1 (WTG1)	Os08g0537800	Deubiquitinating enzyme	−	Expansion	Huang et al. (2017), Wang et al. (2017)
G protein signaling					
RICE G PROTEIN ALPHA SUBUNIT (RGA1)/DWARF1 (D1)	Os05g0333200	G protein α subunit	+	Proliferation	Ashikari et al. (1999), Fujisawa et al. (1999)
RICE G PROTEIN BETA SUBUNIT (RGB1)	Os03g0669100	G protein β subunit	+	Proliferation	Utsunomiya et al. (2011)
GRAIN SIZE3 (GS3)	Os03g0407400	Homolog of G protein γ subunit	−	Proliferation	Fan et al. (2006), Mao et al. (2010)
DENSE AND ERECT PANICLE1 (DEP1)	Os09g0441900	Homolog of G protein γ subunit	−	Proliferation	Huang et al. (2009), Zhou et al. (2009)
MAPK signaling					
SMALL GRAIN1 (SMG1)	Os02g0787300	Mitogen-activated protein kinase kinase 4 (OsMKK4)	+	Proliferation	Duan et al. (2014)
MITOGEN-ACTIVATED PROTEIN KINASE KINASE 6 (OsMAPK6)	Os06g0154500	Mitogen-activated protein kinase 6	+	Proliferation	Liu et al. (2015b)
Phytohormones					
BRASSINOSTEROID-INSENSITIVE 1 (OsBRI1)/DWARF61 (D61)	Os01g0718300	Leucine-rich repeat receptor-like kinase (LRR-RLK); BR receptor	+		Morinaka et al. (2006)
BRI1-ASSOCIATED RECEPTOR KINASE (OsBAK1)	Os08g0174700	Leucine-rich repeat receptor-like kinase (LRR-RLK); BR receptor	+		Yuan et al. (2017)
DWARF 11 (D11)	Os04g0469800	CYP724B1; involved in BR biosynthesis	+	Expansion	Tanabe et al. (2005), Wu et al. (2016)
DWARF 2 (D2)	Os01g0197100		+	Expansion	Fang et al. (2016), Hong et al. (2005)
SLENDER GRAIN (SLG)	Os08g0562500	BAHD acyltransferase-like protein	+	Expansion	Feng et al. (2016)
BRASSINAZOLE-RESISTANT1 (OsBZR1)	Os07g0580500	Transcription factor mediating BR responses	+		Zhu et al. (2015)
GRAIN SIZE 5 (GS5)	Os05g0158500	Putative serine carboxypeptidase	+	Mainly proliferation	Li et al. (2011a, b), Xu et al. (2015a)
GSK3/SHAGGY-LIKE KINASE 2 (GSK2)	Os05g0207500	GSK3/SHAGGY-like; regulator of BR signaling kinase	−		Tong et al. (2012)
DWARF AND LOW-TILLERING (DLT)	Os06g0127800	GRAS family protein	−		Tong et al. (2012)
GRAIN LENGTH 3 (qGL3/GL3.1)	Os03g0646900	Protein phosphatase kelch family-Ser/Thr phosphatase (PPKL)	−	Proliferation	Hu et al. (2012), Qi et al. (2012), Zhang et al. (2012)
BRASSINOSTEROID UPREGULATED1 (BU1)	Os06g0226500	Basic helix-loop-helix (bHLH) transcriptional activator	+		Tanaka et al. (2009)

Table 1 (continued)

Protein name	Accession number	Protein category	Positive (+)/negative (−) regulator	Affecting cell proliferation/expansion	References
BRASSINOSTEROID UPREGULATED 1-LIKE1 (OsBUL1)	Os02g0747900	bHLH transcriptional activator	+	Expansion	Heang and Sassa (2012b), Jang et al. (2017), Jang and Li (2017)
GSE5/SEED WIDTH ON CHROMOSOME 5 (qSW5/GW5)	Os05g0187500	A plasma membrane-associated protein with IQ calmodulin-binding motifs	−	Proliferation	Duan et al. (2017), Liu et al. (2017), Shomura et al. (2008), Weng et al. (2008)
POSITIVE REGULATOR OF GRAIN LENGTH 1 (PGL1)	Os03g0171300	bHLH protein	+	Expansion	Heang and Sassa (2012a)
ANTAGONIST OF PGL1 (APG)	Os05g0139100	bHLH protein	−	Expansion	Heang and Sassa (2012a)
SHORT GRAIN1 (SG1)	Os09g0459200	Unknown function	−	Expansion	Nakagawa et al. (2012)
THOUSAND-GRAIN WEIGHT (TGW6)	Os06g0623700	IAA-glucose hydrolase	−	−	Ishimaru et al. (2013)
BIG GRAIN1 (BG1)	Os03g0175800	Membrane-localized protein	+	Both	Liu et al. (2015a)
SMALL ORGAN SIZE1 (SMOS1)	Os05g0389000	Unusual APETALA2 (AP2)-type transcription factor	+	Expansion	Aya et al. (2014), Hirano et al. (2017)
STRESS_TOLERANCE AND GRAIN_LENGTH (OsSGL)	Os02g0134200	DUF1645 family protein	+	Both	Wang et al. (2016)
Transcription regulators					
GRAIN LENGTH 7 (GL7/GW7/SLG7)	Os07g0603300	TON1 RECRUITING MOTIF (TRM)-containing protein	+		Wang et al. (2015a, b), Zhou et al. (2015)
GRAIN SIZE 2 (GS2)	Os02g0701300	Rice growth-Regulating Factor 4 (OsGRF4)	+	Mainly expansion	Che et al. (2015), Duan et al. (2015), Hu et al. (2015), Li et al. (2016), Sun et al. (2016)
GROWTH-REGULATING FACTOR-INTERACTING FACTOR 1 (OsGIF1)	Os03g0733600	Rice GRF-interacting protein 1	+	Mainly expansion	Duan et al. (2015), He et al. (2017), Li et al. (2016)
GRAIN LENGTH AND WIEIGHT 7 (GLW7)	Os07g0505200	Squamosa promoter binding protein like13 (OsSPL13)	+	Expansion	Si et al. (2016)
GRAIN WIDTH 8 (GW8)	Os08g0531600	Squamosa promoter binding protein like16 (OsSPL16)	+	Proliferation	Wang et al. (2015a, 2012)
GRAIN WEIGHT 6a (GW6a)	Os06g0650300	Histone H4 acetyltransferase	+	Proliferation	Song et al. (2015)
GRAIN LENGTH 4 (GL4)	ORGLA04G0254300	Myb-like protein	+	Expansion	Wu et al. (2017)
Other factors					
GIANT EMBRYO (GE)/BIG GRAIN2 (BG2)/GRAIN LENGTH 3.2 (GL3.2)	Os07g0603700	CYP78A13	+		Nagasawa et al. (2013), Xu et al. (2015b), Yang et al. (2013)
CYP704A3	Os04g0573900	CYP704A3	+		Tang et al. (2016)
GRAIN NUMBER, GRAIN LENGTH AND AWN DEVELOPMENT1 (GAD1)/REGULATOR OF AWN ELONGATION 2 (RAE2)	Os08g0485500	EPIDERMAL PATTERNING FACTOR-LIKE family peptide	+	Proliferation	Bessho-Uehara et al. (2016), Jin et al. (2016)

Table 1 (continued)

Protein name	Accession number	Protein category	Positive (+)/negative (−) regulator	Affecting cell proliferation/expansion	References
FUWA	Os02g0234200	NHL domain-containing protein	−	Proliferation	Chen et al. (2015)
SMALL AND ROUND SEED 3 (SRS3)/SMALL AND ROUND GRAINS (SAR1)/OsKINESIN-13A	Os05g0154700	OsKinesin-13A	+	Expansion	Deng et al. (2015), Kitagawa et al. (2010)
DENSE AND ERECT PANICLE 2 (DEP2)/SMALL AND ROUND SEED 1 (SRS1)/ERECT PANICLE2-1 (EP2-1)	Os07g0616000	Endoplasmic reticulum-localized protein	+	Both	Abe et al. (2010), Li et al. (2010), Zhu et al. (2010)
ABC1-LIKE KINASE RELATED TO GRAIN SIZE AND WEIGHT (OsAGSW1)	Os05g0323800	Chloroplast-localized ABC1 protein kinase	+	Proliferation	Li and Li (2015)
SMALL AND ROUND SEED 5 (SRS5)	Os11g0247300	Alpha-tubulin	+	Expansion	Segami et al. (2012)

by Minghui 63 is associated with the long-grain varieties widely cultivated in the world, while the *gs3*^{del357} related to super short grains is extremely rare, suggesting that the *GS3* has been selected during rice breeding.

GS3 shares some homology with DENSE AND ERECT PANICLE1 (DEP1), which is encoded by the QTL locus *DEP1*/*qPE9-1*. *DEP1*/*qPE9-1* is characterized as a major rice grain yield QTL for panicle architecture (Huang et al. 2009; Zhou et al. 2009). DEP1 contains the N-terminal region (ORS domain), the putative transmembrane domain, and the C-terminal 4-disulfide-core domain. A gain-of-function mutation at the *DEP1* locus results in truncation of the ORS domain, which is very similar to the gain-of-function mutation in the *GS3* allele in Chuan 7. This mutant *DEP1* allele enhances meristematic activity, resulting in dense and erect panicles, increased grain number per panicle and increased grain yield (Huang et al. 2009).

The N-terminal domain (ORS domain) of GS3 and DEP1 shares significant sequence similarity with the N-terminal domain of *Arabidopsis* heterotrimeric G protein γ-subunits (Gγ) AGG1 and AGG2, and atypical Gγ AGG3, and therefore was considered as γ-like domains (Chakravorty et al. 2011; Huang et al. 2009; Li et al. 2012b; Mao et al. 2010). In *Arabidopsis*, AGG3 interacts with Gβ (AGB1), and the role of AGG3 in seed growth is dependent on Gα and Gβ, indicating that these three G proteins function in a same genetic pathway to control seed growth (Li et al. 2012a, b). Loss of function of Gα (RGA1) or suppression of Gβ (RGB1) decreases grain size in rice (Ashikari et al. 1999; Fujisawa et al. 1999; Utsunomiya et al. 2011), suggesting that growth of rice grain is also regulated by Gα and Gβ. The N-terminal domains of GS3 and DEP1 contain several conserved residues critical for binding of Gβ subunit, although it is still unclear whether GS3 and DEP1 act with RGA and RGB1 to regulate rice grain size. Intriguingly, rice GS3 and DEP1 play negative roles in grain size, while AGG3 positively regulates seed growth in *Arabidopsis* (Li et al. 2012a). It is unclear whether they function with different cofactors or act on different downstream components to control seed development. Further studies would be expected to elucidate why Gγ proteins have different effects on seed growth in *Arabidopsis* and rice.

Control of grain size by the MAPK signaling pathway

The mitogen-activated protein kinase (MAPK) cascades are evolutionary conserved signaling modules in eukaryotes and play critical roles in transducing developmental and defense signals in plants (Meng and Zhang 2013; Xu and Zhang 2015). Recent studies found that the MAPK cascades are also involved in grain size control. Loss of function of

SMALL GRAIN 1 (SMG1)/MITOGEN-ACTIVATED PROTEIN KINASE KINASE4 (OsMKK4) results in small grains due to decreased cell number in spikelet hulls, suggesting that OsMKK4 promotes grain growth (Duan et al. 2014). Similarly, OsMAPK6 also acts positively in grain size control (Liu et al. 2015b). The mutation in *OsMAPK6* restricts cell proliferation in spikelet hulls, leading to small grains. OsMKK4 interacts with OsMAPK6 and phosphorylates OsMAPK6, suggesting that OsMKK4 and OsMAPK6 act as a module to control grain size (Kishi-Kaboshi et al. 2010; Liu et al. 2015b). Interestingly, both OsMKK4 and OsMAPK6 affect brassinosteroid (BR) responses and the expression of BR-related genes, suggesting a possible link between BR signaling and the MAPK pathways (Duan et al. 2014; Liu et al. 2015b). It would be worthwhile to identify the upstream MAPKKK and the downstream components of OsMKK4-OsMAPK6 in grain size control.

Control of grain size by the ubiquitin–proteasome pathway

Modification of target proteins by ubiquitin chains is an important regulatory process in eukaryotes. Ubiquitination requires the sequential action of three enzymes: the ubiquitin-activating enzyme (E1), the ubiquitin-conjugating enzyme (E2), and the ubiquitin ligase (E3). The diverse length and linkage of the ubiquitin chains have different effects on the target protein. Generally, linkage via K48 leads to protein degradation by the 26S proteasome. The ubiquitin chain can be removed from substrate proteins by the deubiquitinating enzymes (DUBs). Recent studies showed that the ubiquitin–proteasome pathway plays important roles in seed size control.

GW2

The major QTL for grain width (*GW2*) was identified by map-based cloning using the progeny of a cross between *japonica* variety WY3 (large grain) and *indica* variety Fengaizhan-1 (FAZ1; small grain) (Song et al. 2007). *GW2* encodes a RING-type E3 ubiquitin ligase. The WY3 allele harbors a 1-bp deletion in the coding region of *GW2*, leading to a premature stop codon. This loss-of-function mutation enhances cell proliferation in the spikelet hulls and accelerates grain filling, resulting in increased grain width, weight, and yield. Further analysis revealed that another wide grain variety Oochikara carries the same *GW2* allele as WY3. Importantly, the *GW2* allele in WY3 could increase grain size and yield with little effect on appearance and no reduction in cooking or eating quality and thus could be a useful target in rice breeding.

GW2 shares significant sequence similarity with *Arabidopsis* E3 ubiquitin ligase DA2, which interacts with the ubiquitin-receptor DA1 to control seed size (Li et al. 2008; Xia et al. 2013). Recent studies show that DA2 can ubiquitylate DA1 to active its peptidase activity, and the activated DA1 then cleaves downstream substrates to control seed and organ growth (Dong et al. 2017). These studies provide some clues to the molecular mechanisms by which GW2 controls grain growth. It would be challenging but interesting to identify the substrates and downstream components of GW2 in grain size control.

OsOTUB1/WTG1

Loss of function of the deubiquitinating enzyme OsOTUB1/WIDE AND THICK GRAIN 1 (WTG1) increases grain width, grain thickness, and grain number per panicle (Huang et al. 2017). OsOTUB1 controls grain size and shape mainly by influencing cell expansion. Overexpression of *OsOTUB1* results in narrow, thin, and long grains. Later, *OsOTUB1* is also identified as a major QTL determining the 'new plant type' (NPT) architecture, which is characterized by larger panicles, stronger culms, and fewer sterile tillers (Wang et al. 2017). OsOTUB1 interacts with OsSPL14/IPA1 that is an important regulator of ideal plant architecture. This interaction limits K63-linked ubiquitination (K63Ub) of OsSPL14 and in turn promotes K48Ub-dependent proteasome degradation of OsSPL14. Downregulation of *OsOTUB1* causes accumulation of OsSPL14 and results in the NPT architecture. It will be interesting to investigate whether OsOTUB1 regulates grain size through SPL transcription factors.

Control of grain size by phytohormones

Brassinosteroids

Brassinosteroids (BRs) are a class of polyhydroxysteroid plant hormones that are essential for the proper regulation of multiple physiological processes during plant growth and development (Clouse 2011). The role of BR on grain size control has been shown by a number of studies. The BR-deficient mutants *dwarf11* and *dwarf2* produce small and short grains, suggesting that BR promotes grain growth (Fang et al. 2016; Hong et al. 2005; Tanabe et al. 2005; Wu et al. 2016). Consistently, some regulators of BR homeostasis have effects on grain size. For example, enhanced expression of *SLENDER GRAIN (SLG)* causes elevated BR contents, leading to long and narrow grains (Feng et al. 2016), whereas loss of function of *XIAO* results in typical BR-related phenotypes and reduced grain length (Jiang et al. 2012) .

BRs are perceived by the membrane-localized receptor kinase BRASSINOSTEROID-INSENSITIVE1 (BRI1) and its partner BRI1-ASSOCIATED RECEPTOR KINASE (BAK1). BR signal then initiates a cascade of cellular events, leading to inactivation of BRASSINOSTEROID-INSENSITIVE 2 (BIN2) and activation of the two transcription factors BRASSINAZOLE-RESISTANT1 (BZR1) and BZR2 for transcription of downstream genes (Clouse 2011). In rice, loss of function of OsBRI1 or OsBAK1 results in BR-insensitive phenotypes and small grains (Morinaka et al. 2006; Yuan et al. 2017), whereas overexpression of OsBZR1 increases grain length, grain width and grain weight (Zhu et al. 2015). The downstream components of BR signaling also affect grain size. The rice counterpart of BIN2, GSK2, negatively regulates grain size (Tong et al. 2012). GSK2 can phosphorylate the GRAS family protein DWARF AND LOW-TILLERING (DLT/OsGRAS-32/D62/GS6), a positive regulator that mediates several BR responses in rice (Tong et al. 2012). One of the studies showed that *d62* has short and wide grains (Li et al. 2010b), while another study showed that the grain width of *gs6* was increased but the grain length was not significantly different compared with the wild-type 93-11 (Sun et al. 2013).

Several regulators of BR signaling were also involved in grain size control. A pair of bHLH proteins POSITIVE REGULATOR OF GRAIN LENGTH 1 (PGL1) and ANTAGONIST OF PGL1 (APG) antagonistically regulate rice grain length and weight by controlling cell elongation in lemma/palea through heterodimerization (Heang and Sassa 2012a). APG negatively regulates grain length, while its function is inhibited by PGL1. Overexpression of *PGL1* or suppression of *APG* results in increased BL sensitivity and long grains, suggesting that PGL1 and APG1 control grain length probably by mediating BR signaling. Similar to PGL1, the atypical bHLH protein PGL2/BRASSINOSTEROID UPREGULATED 1-LIKE1 (OsBUL1) promotes grain length by suppressing the function of APG (Heang and Sassa 2012b). PGL2/OsBUL1 can also form a transcriptional activator complex with another basic helix-loop-helix (bHLH) transcriptional activator OsBUL1 COMPLEX1 (OsBC1) and KxDL motif-containing protein LO9-177 to regulate BR response and grain size (Jang et al. 2017; Jang and Li 2017). The closet homolog of PGL2/OsBUL1, BRASSINOSTEROID UPREGULATED1 (BU1), is also a positively regulator of BR response and grain size (Heang and Sassa 2012b; Tanaka et al. 2009). However, no interaction was detected between BU1 and APG, indicating that BU1 might control grain length independently of APG (Heang and Sassa 2012b). In addition, SHORT GRAIN1 (SG1), a protein with unknown function, acts as a negative regulator of BR response and grain size (Nakagawa et al. 2012). Overexpression of *SG1* in rice causes brassinosteroid (BR)-deficient phenotype and short grains, while downregulation of *SG1* and *SG1-LIKE PROTEIN1* results in long grains.

The QTL for grain size *GRAIN SIZE 5* (*GS5*) encodes a putative serine carboxypeptidase which functions as a positive regulator of grain size (Li et al. 2011b). *GS5* was identified by using a double haploid (DH) population (92 lines) derived from a cross between Zhenshan 97 (wide grains) and H94 (slender grains). Higher expression of *GS5* increases grain width and grain yield by accelerating cell division and cell expansion in the spikelet hull. Sequence analysis of 51 rice accessions from a wide geographic range revealed that polymorphisms in the promoter region of *GS5* are likely correlated with grain width, indicating that natural variation in *GS5* contributes to grain size diversity in rice. Furthermore, a recent study found that GS5 regulates grain size by preventing OsBAK1-7 endocytosis and enhancing BR signaling, suggesting a possible link between GS5 and BR signaling in grain size control (Xu et al. 2015a).

The major QTL for grain length (*qGL3/qGL3.1*) was identified by three independent studies (Hu et al. 2012; Qi et al. 2012; Zhang et al. 2012). *qGL3/GL3.1* encodes a Ser/Thr phosphatase with Kelch-like repeat domain (OsPPKL1). OsPPKL1 controls cell division in the spikelet by directly dephosphorylating Cyclin-T1;3. A single nucleotide transition from C to A (c. + 1092C → A) causes an aspartate to glutamate change (Asp364Glu) in a conserved AVLDT motif of the second Kelch domain in OsPPKL1, resulting in weaker dephosphorylation activity. The *qgl3* allele increases grain length and grain yield without affecting grain quality. Sequencing analysis of the *qGL3* locus using 94 rice germplasms showed that only one variety (DT108) carries the (c. + 1092C → A) transition, suggesting that *qgl3* is a rare allele. Furthermore, the *qgl3* allele could significantly increase grain yield in various rice varieties. Therefore, it could be used in breeding elite rice varieties (Zhang et al. 2012).

There are two OsPPKL1 homologs in rice, OsPPKL2 and OsPPKL3 (Zhang et al. 2012). Interestingly, OsPPKL1 and OsPPKL3 limit grain length, while OsPPKL2 promotes grain growth. OsPPKL2 belongs to a subgroup with *Arabidopsis* homologs AtBSU1 and AtBSL1, two serine–threonine protein phosphatases that function in brassinosteroid signaling pathway to promote cell elongation and cell division. It would be worthwhile to investigate whether OsPPKLs influence grain length through brassinosteroid-mediated signaling.

The *SEED WIDTH ON CHROMOSOME 5* (*GW5/qSW5*) is a major QTL that determines grain-width differences between *indica* and *japonica* varieties (Shomura et al. 2008; Weng et al. 2008). *GW5/qSW5* was identified by two independent studies using different recombinant inbred lines (RILs) generated from crosses between Asominori/Nipponbare (wide grains) and IR24/Kasalath

(slender grains) (Shomura et al. 2008; Weng et al. 2008). Sequencing results revealed that *GW5/qSW5* is associated with a 1212-bp deletion. Transformation of a 11.2-kbp Kasalath fragment covering the deletion region resulted in thin rice grains in Nipponbare background, suggesting that *qSW5* is in this region. One of the predicted ORFs (GenBank: Kasalath *qSW5* gene, AB433345) in the 11.2-kbp region, which encodes an unknown protein, was proposed to be the *qSW5* gene (Shomura et al. 2008), while another study proposed that an ORF (Gene bank: IR24, *GW5*, DQ991205) encoding an ubiquitin-interacting protein is the *GW5* gene (Weng et al. 2008). Thus, GW5 has been proposed to be involved in the proteasome pathway (Li and Li 2014, 2016). However, functional complementation tests using these individual ORFs were lacking. Recent studies revealed that the transformation of Nipponbare with another ORF (LOC_Os05g09520) in the 11.2-kbp Kasalath fragment resulted in thin grains, suggesting that this ORF encodes GW5 (Liu et al. 2017). Meanwhile, this ORF was identified as a major QTL for grain size (*GSE5*) through genome-wide association analysis (Duan et al. 2017). *GSE5/GW5* encodes a plasma membrane-associated protein with IQ calmodulin-binding motifs (Duan et al. 2017; Liu et al. 2017). GSE5/GW5 physically associates with rice calmodulin (OsCAM1), suggesting that calcium signaling may play a role in grain size control. However, how GSE5/GW5 mediates calcium signaling to regulate grain size remains unknown.

Further analysis showed that natural variation in the promoter region of *GSE5* contributes to grain size diversity in cultivated rice (Duan et al. 2017; Liu et al. 2017). *GSE5* has three major haplotypes in cultivated rice: most *japonica* varieties have a 1212-bp deletion (DEL2) in the promote region of *GSE5*; most narrow-grain *indica* varieties have no deletion; and most wide-grain *indica* varieties contain a 950-bp deletion (DEL1) and a 367-bp insertion (IN1) in the promoter region of *GSE5* and a nucleotide change (G/A) in the first exon of *GSE5*. DEL1 in *indica* varieties and DEL2 in *japonica* varieties associate with decreased expression of *GSE5*, resulting in wide grains. The DEL1 and DEL2 deletions likely originated from different wild rice accessions during rice domestication and are widely utilized by rice breeders. Knockout of *GSE5/GW5* using CRISPR/Cas9 technology in *japonica* and *indica* varieties significantly increased grain width and weight, suggesting that the *GSE5/GW5* locus may be used to improve rice yield (Duan et al. 2017; Liu et al. 2017). In addition, GSE5/GW5 can repress the kinase activity of GSK3/SHAGGY-like kinase (GSK2), a component of brassinosteriod (BR) signaling pathway, suggesting that GSE5/GW5 may regulate grain width by modulating BR signaling (Liu et al. 2017). Interestingly, GSE5/GW5 controls grain size by regulating cell proliferation in spikelet hulls, while GSK2 affects grain size predominantly by influencing cell expansion in spikelet hulls.

Auxin

Although auxin has been demonstrated to play important roles in many aspects of plant growth and development, its role in grain size control remains elusive. So far, only a few pieces of evidence show a connection between auxin and grain size control. The major QTL for thousand-grain weight (*TGW6*) was identified by positional cloning using backcrossed inbred lines produced from Nipponbare (heavy grains) and Kasalath (light grains) (Ishimaru et al. 2013). *TGW6* encodes IAA-glucose hydrolase, which regulates the transition from the syncytial to the cellular phase during early endosperm development by regulating IAA supply. A 1-bp deletion in the Kasalath allele causes loss of function of TGW6 and results in increased grain weight and grain yield, suggesting that TGW6 negatively regulates grain growth. The Kasalath *TGW6* allele can also increase the accumulation of carbohydrates before heading and consequently improve yield without change in grain quality. Analysis of different wild rice lines (*Oryza rufipogon*) and 69 rice varieties showed that the Kasalath *TGW6* allele has probably not been selected in rice breeding and thus could be used in rice yield improvement. Notably, TGW6 influences grain length and grain weight with no effect on husk size, indicating a distinct regulation mechanism from other grain size regulators.

BIG GRAIN1 (BG1), a novel membrane-localized protein, was identified as a positive regulator of grain size (Liu et al. 2015a). Activation of *BG1* increases grain size and grain weight due to increased cell proliferation and cell expansion in spikelet hulls, whereas suppression of *BG1* results in small grains. BG1 affects auxin response and transport, suggesting that it may control grain growth through auxin signaling. Nonetheless, future studies need to elucidate the function of BG1 in auxin transport/signaling.

Loss of function of SMALL ORGAN SIZE1 (SMOS1), an unusual APETALA2 (AP2)-type transcription factor, results in small grains and organs (Aya et al. 2014). SMOS1 promotes cell expansion and microtubule orientation. The promoter region of *SMOS1* gene contains auxin response elements (AuxRE), and the expression of *SMOS1* was induced by exogenous auxin treatment, suggesting that SMOS1 acts as an auxin-dependent regulator for cell expansion. SMOS1 directly regulates the expression of *PHOSPHATEINDUCED PROTEIN 1* (*OsPHI-1*) that is involved in cell expansion. In addition, a recently report showed that SMOS1 forms a complex with DLT to integrate auxin and brassinosteroid signaling in rice (Hirano et al. 2017). However, the genetic relationship between SMOS1 and DLT in grain size control is still unclear.

Cytokinin

Recent findings suggest that cytokinin plays important roles in controlling grain number and grain size. A major QTL for grain number, *Gn1a*, encodes cytokinin oxidase/dehydrogenase (OsCKX2) that modulates cytokinin accumulation by catalyzing the degradation of active CKs (Ashikari et al. 2005). Reduced expression of *OsCKX2* increases grain number, with no effects on grain size. Thus, *Gn1* could be used for increasing grain yield. The expression of *OsCKX2* is regulated by LARGE PANICLE (LP) and DROUGHT AND SALT TOLERANCE (DST) (Li et al. 2011a, 2013). LARGER PANICLE (LP) is a Kelch repeat-containing F-box protein localized in endoplasmic reticulum (ER). The *lp* mutants show increased grain number, grain size and grain yield. *OsCKX2* was downregulated in the *lp* mutants, implying that LP might regulate grain number and grain size by modulating cytokinin level (Li et al. 2011a). DST is a zinc finger transcription factor. It directly regulates OsCKX2 expression in the reproductive meristem, thereby influencing the number of the reproductive organs through modulating CK accumulation (Li et al. 2013). The semidominant DST^{reg1} allele affects DST-directed regulation of *OsCKX2* expression, leading to elevated CK levels in the inflorescence meristem and increased grain number, grain weight and grain yield (Li et al. 2013).

STRESS_tolerance and GRAIN_LENGTH (*OsSGL*), an abiotic stress-induced gene, has been involved in stress tolerance and grain length (Wang et al. 2016). *OsSGL* encodes a putative DUF1645 family protein with unknown function. Overexpression of *OsSGL* not only enhances drought tolerance but also increases grain length, grain weight and grain number per panicle, resulting in a significant increase in yield. *OsSGL* promotes grain growth by increasing longitudinal cell number and cell size in the lemma/palea. Transcriptome analysis showed that elevated expression of *OsSGL* alters the expression of several genes related to CK signaling process, suggesting that *OsSGL* may regulate grain length and stress response through modulating CK signal transduction.

An-2 is a QTL locus for awn length. The *An-2* gene encodes a Lonely Guy (LOG) homologous enzyme that catalyzes the last step of cytokinin synthesis. An-2 promotes awn elongation by enhancing cell division, but decreases grain production by reducing grain weight and grain number. Genetic variation analysis shows that the cultivar allele of *An-2* shows significantly reduced nucleotide diversity compared with wild rice, indicating that this locus was selected for reduced awn length and increased grain yield during rice domestication (Gu et al. 2015).

Control of grain size by transcriptional regulatory factors

GLW7

The major QTL for grain length and weight (*GLW7*) was identified by an approach integrating genome-wide association testing with functional analysis on grain size in a population of 381 *japonica* varieties (Si et al. 2016). *GLW7* encodes the plant-specific transcription factor SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 13 (OsSPL13). OsSPL13 positively regulates grain length and yield by promoting cell expansion in the grain hull. Higher expression of *OsSPL13* is associated with large grains in tropical *japonica* rice due to difference in the 5′ UTR sequence of *OsSPL13* which affects transcription and translation. This large-grain allele of *GLW7* in tropical *japonica* rice was introgressed from *indica* varieties under artificial selection. Furthermore, GLW7 directly associates with the promoter region of *SMALL AND ROUND SEED 5* (*SRS5*) and promotes its expression. *SRS5* encodes alpha-tubulin protein (Segami et al. 2012). A semidominant mutation of *SRS5* leads to short grains with reduced cell length (Segami et al. 2012), while plants overexpressing *SRS5* form long grains (Segami et al. 2017). However, the genetic relationship between *GLW7* and *SRS5* remains unclear.

GW8

The QTL for grain width (*GW8*) was identified by analysis of segment substitution lines (SSSLs) from a cross between Basmati385 (slender grain) and HJX74 (wide grain) (Wang et al. 2012). *GW8* encodes OsSPL16, which increases grain width and yield by promoting cell division and grain filling (Wang et al. 2012). In Basmati rice, a 10-bp deletion in the deletion in the *OsSPL16* promoter region results in reduced transcription and leads to slender grains and better quality of appearance. Haplotype diversity of the *OsSPL16* sequence suggests that the Basmati haplotype was selected due to its association with better grain quality, whereas in elite *indica* varieties the HJX74 haplotype was selected for higher grain productivity.

The major QTL for rice grain length (*GL7*)/*GRAIN WIDTH 7* (*GW7*)/*SLENDER GRAIN ON CHROMOSOME 7* (*SLG7*) was identified by three independent studies (Wang et al. 2015a, b; Zhou et al. 2015). *GL7*/*GW7*/*SLG7* encodes a TON1 RECRUITING MOTIF (TRM)-containing protein homologous to *Arabidopsis* LONGIFOLIA proteins involved in microtubule regulation. In long-grain varieties, elevated expression of *GL7*

leads to slender grains (Wang et al. 2015a, b), and this beneficial allele of *GL7* has been selected in rice breeding (Wang et al. 2015b). One of the studies showed that tandem duplication of a 17.1-kb segment at the *GL7* locus leads to upregulation of *GL7*, resulting in increased grain length and improvement in grain appearance quality (Wang et al. 2015b). By contrast, another study reported that the mutation in the promoter region of *GL7* causes its high expression (Wang et al. 2015a). The expression of *GL7*/*GW7*/*SLG7* was regulated by the SPL transcription factor OsSPL16, which is encoded by the QTL for grain width (*GW8*) (Wang et al. 2015a). It was shown that OsSPL16/GW8 binds to the promoter of *GL7*/*GW7*/*SLG7* and represses its transcription to regulate cell proliferation in the spikelet hull (Wang et al. 2015a). Controversially, two studies demonstrated that *GL7*/*GW7*/*SLG7* regulates grain length through cell expansion. It enhances cell elongation in the grain-length direction and restricts cell expansion in the grain-width direction (Wang et al. 2015b; Zhou et al. 2015). Further studies need to clarify whether *GL7*/*GW7*/*SLG7* controls grain size through cell proliferation or cell expansion.

GS2/*GL2*/*GLW2*/*PT2*

GRAIN SIZE 2 (*GS2*)/*GRAIN-LENGTH-ASSOCIATED* (*GL2*)/*GRAIN LENGTH AND WIDTH 2* (*GLW2*)/*PANICLE TRAITS 2* (*PT2*), a major QTL for grain length, grain width and weight, was identified independently by five research groups using different F2 populations (Che et al. 2015; Duan et al. 2015; Hu et al. 2015; Li et al. 2016; Sun et al. 2016). *GS2* encodes a plant-specific transcription factor GROWTH-REGULATING FACTOR 4 (OsGRF4), which regulates grain size through predominantly increasing cell expansion and slightly promoting cell proliferation in the spikelet hull. The expression of *OsGRF4* is regulated by miRNA396. In large-grain varieties, a 2-bp substitution mutation (TC → AA, $GS2^{AA}$) in miR396 targeting site of *OsGRF4* perturbs OsmiR396-directed regulation, causing elevated expression of *OsGRF4*, and resulting in large and heavy grains and increased grain yield. Sequence analysis of various cultivars revealed that the $GS2^{AA}$ allele is a rare allele and has not been selected by breeders, suggesting that the $GS2^{AA}$ allele could be used to increase grain size and yield (Duan et al. 2015; Hu et al. 2015; Sun et al. 2016).

OsGRF4 interacts with the transcription coactivators GRF-INTERACTING FACTOR 1/2/3 (OsGIF1/2/3) (Duan et al. 2015; Li et al. 2016). Overexpression of *OsGIF1* increases grain size and weight in rice (Che et al. 2015; Duan et al. 2015; He et al. 2017; Li et al. 2016). Thus, the OsmiR396–OsGRF4–OsGIFs regulatory module plays important roles in grain size control. In addition, OsGRF4 interacts with GSK2 which functions in BR signaling (Che et al. 2015). GSK2 can repress the transcription activation activity of OsGRF4 and suppress the function of OsGRF4 in grain size control, suggesting that OsGRF4 may function with BR signaling to regulate grain size.

GW6a

The QTL for grain weight (*GW6a*) was detected using a set of backcrossed inbred lines derived from a cross of Kasalath (light grains) with Nipponbare (heavy grains) (Song et al. 2015). *GW6a* encodes a new-type GNAT-like protein with intrinsic histone acetyltransferase activity (OsglHAT1). OsglHAT1 is localized in nucleus and functions presumably via regulation of gene expression. Elevated *OsglHAT1* expression increases grain weight and grain yield by enhancing cell proliferation in spikelet hulls and accelerating grain filling. *GW6a* is the first QTL for yield component traits that encodes a chromatin modifier. Importantly, *GW6a* has not been selected during rice domestication and modern breeding, indicating that it could be exploited in rice yield improvement.

GL4

The quantitative trait locus for grain length (*GL4*) in African rice was detected using F2 population derived from a cross between introgression line GIL25 (long grains) and a cultivar of African cultivated rice IRGC102305 (short grains) (Wu et al. 2017). *GL4* encodes a Myb-like protein sharing highly identity with SH4/SHA1, its orthologue in Asian wild rice. SH4 was previously reported to control seed shattering, and the *Ossh4* allele resulting with non-shattering has been selected during the domestication of Asian cultivated rice *O. sativa*. GL4 regulates grain length in African cultivated rice (*O. glaberrima*) by promoting longitudinal cell elongation in the glumes. Like SH4/SHA1 in Asian wild rice, *GL4* also controls seed shattering. A single nucleotide substitution (C760T) in the IRGC102305 allele causes a premature stop codon, leading to small grains and loss of grain shattering during African rice domestication. By contrast, GIL25 harboring the wild-type *GL4* allele has increased grain length and grain yield. Further studies showed that *GL4*/*SH4* is a key domestication gene with pleiotropic effects, which controls both grain size and shattering in rice. During crop domestication, increasing seed size and reducing seed shattering were two main selection targets. *Ossh4* and *Ogsh4* were selected in parallel during the domestication of Asian and African rice, resulting in loss of grain shattering. The *Ossh4* mutation does not change the seed size in Asian cultivated rice, while *Ogsh4* leads to small seeds in African cultivated rice. Replacing the *Ogsh4* allele with the *Ossh4* allele would enhance the grain yield of *O. glaberrima*.

Other factors in grain size control

P450 family proteins

The CYP78A subfamily P450 monooxygenase GIANT EMBRYO (GE)/BIG GRAIN2 (BG2)/GRAIN LENGTH 3.2 (GL3.2) is critical for coordinating rice embryo and endosperm development. The *GE/BG2* gene was identified by three independent studies (Nagasawa et al. 2013; Xu et al. 2015b; Yang et al. 2013). It is expressed predominantly in the scutellar epithelium, the interface region between embryo and endosperm, and coordinates the development of the embryo and endosperm (Nagasawa et al. 2013; Yang et al. 2013). Loss of function of *GE* leads to large embryos and small endosperm, whereas *GE* overexpression causes small embryos and enlarged endosperm, suggesting that GE is crucial for the coordinated development of the embryo and the endosperm.

Another putative cytochrome P450, CYP704A3, is also responsible for grain length (Tang et al. 2016). The expression of *CYP704A3* was regulated by miRNA. A SNP at the miRNA binding site in the 3′-UTR region of *CYP704A3* is associated with rice grain size. Downregulation of *CYP704A3* via RNAi increases grain length.

Cytochrome P450s play important roles in a variety of biosynthetic pathways. Recently, several cytochrome P450s have been involved in seed size control. In *Arabidopsis*, KLUH (KLU)/CYP78A5 promotes seed and organ growth in a non-cell-autonomous manner (Adamski et al. 2009; Anastasiou et al. 2007; Eriksson et al. 2010; Wang et al. 2008). It was proposed that KLU can generate a mobile growth signal that is distinct from the classic phytohormones (Anastasiou et al. 2007). The expression of *KLU* is regulated by the NGATHA-like B3 domain transcriptional repressor (NGAL2)/SUPPRESSOR OF DA1 (SOD7) and its homolog NGAL3/DEVELOPMENT-RELATED PCG TARGET IN THE APEX 4 (DPA4). NGAL2/SOD7 directly binds to the promoter of KLUH (KLU)/CYP78A5 and represses the transcription of *KLU* to regulate seed growth. Two homologs of KLU, EOD3/CYP78A6 and CYP78A9, control seed size in *Arabidopsis* by promoting both cell proliferation and cell expansion in maternal integuments.

GAD1

GRAIN NUMBER, GRAIN LENGTH AND AWN DEVELOPMENT1 (GAD1)/REGULATOR OF AWN ELONGATION 2 (RAE2) was identified by two independent studies (Bessho-Uehara et al. 2016; Jin et al. 2016). GAD1/RAE2 encodes a small secretary signal peptide belonging to the EPIDERMAL PATTERNING FACTOR-LIKE family. It promotes grain elongation and awn development by enhancing cell division at the apices of glumes. Loss of function of *GAD1* results in increased number of grains per panicle, short grains, and awnless phenotype. The GAD1/RAE2 precursor is specifically cleaved by its requisite processing enzymes, SUBTILISIN-LIKE PROTEASE 1 (SLP1), in the rice spikelet (Bessho-Uehara et al. 2016). EPF/EPFL family members have been shown to regulate multiple biological processes in plants (Murphy et al. 2012). In *Arabidopsis*, the EPFL family peptides bind to the membrane-bond ERECTA family receptors, which transduce the signals through MAPK cascade to regulate stomata development (Bergmann and Sack 2007; Lampard et al. 2009; Lee et al. 2012, 2015; Pillitteri and Dong 2013). It would be interesting to identify the receptor for GAD1 and investigate whether the OsMKK4-OsMPK6 module acts in a same pathway with GAD1 to regulate grain size in the future.

FUWA

The *fuwa* mutant shows compact plant architecture with wide, thick and short grains (Chen et al. 2015). *FUWA* encodes an NHL domain-containing protein that is evolutionary conserved. Downregulation of *FUWA* results in erect panicles and increased grain size in both *indica* and *japonica* rice, suggesting a potential approach to improve agronomic traits. Several cyclins and cyclin-dependent kinases were upregulated in the *fuwa* mutant, suggesting that FUWA controls grain growth by regulating cell-cycle progression. The detailed mechanisms of FUWA in grain size control remains to be further investigated.

OsKinesin-13A

SMALL AND ROUND SEED 3 (SRS3)/SMALL AND ROUND GRAINS (SAR1)/OsKINESIN-13A is an active microtubule depolymerase, which mainly distributes on vesicles derived from the Golgi apparatus and is destined for the cell surface (Deng et al. 2015). Loss of function of *OsKinesin-13A* leads to short grains due to decreased cell elongation in the glumes (Deng et al. 2015; Kitagawa et al. 2010). The *srs3* mutant shows defective orientation of cellulose microfibrils and microtubule turnover, suggesting that OsKinesin-13A may control cell elongation and grain length through affecting cellulose microfibril orientation and vesicle transport (Deng et al. 2015).

DEP2/SRS1

DENSE AND ERECT PANICLE 2 (DEP2)/SMALL AND ROUND SEED 1 (SRS1)/ERECT PANICLE2-1 (EP2-1) is

involved in the control of both panicle architecture and grain size (Abe et al. 2010; Li et al. 2010a; Zhu et al. 2010). The *dep2* mutant has dense and erect panicles as well as small and round grains. *DEP2* encodes an endoplasmic reticulum-localized protein without any known functional domain. The reduced grain length of *srs1-1* is due to the reduction in both cell length and cell number in the longitudinal direction, and the elongation of the cells in the lateral direction of the lemma (Abe et al. 2010). Interestingly, although the *dep2* mutant has a compact plant architecture, the grain production is comparable to that of the wild type, indicating that this allele has important implications for rice breeding.

OsAGSW1

A chloroplast-localized ABC1 protein kinase, OsAGSW1 (ABC1-like kinase related to grain size and weight), is involved in the regulation of grain size and weight. OsAGSW1 promotes grain growth by regulating the number of external parenchyma cells. Overexpression of *OsAGSW1* increases the number of external parenchyma cells in the spikelet hull, leading to increased grain size, grain weight, grain filling rate and 1000-grain weight. However, the molecular mechanisms by which OsAGSW1 controls grain growth are still unclear.

Discussion and perspective

In the past decades, there have been great research progresses on grain size control in rice. A number of grain size regulators related to several signaling pathways were identified (Fig. 2, Table 1). However, our understanding of the mechanisms of grain size control is just beginning and full of gaps. The molecular roles of some regulators in grain size control are still unclear or controversial. The genetic relationships between different regulators and the molecular interactions between different signaling pathways are largely unknown. In previous studies, the mutant alleles used by independent research groups were usually in different genetic backgrounds, which might lead to inconsistent conclusions. Besides, the near-isogenic lines used for genetic analyses might contain other mutations. These problems can now be resolved by newly emerging genome-editing technologies, including CRISPR/Cas9, which allow researchers to knock out candidate genes and analyze their genetic interactions in the same genetic background. In addition, system biology and new biotechnologies will facilitate the studies on grain size control. For instants, the genome-wide association study and modern omics analysis could help to identify novel grain size regulators. Future challenges are to elucidate the molecular mechanisms of identified regulators in grain growth control, identify novel regulators to fill up the gaps in each signaling pathway, and build up genetic frameworks regulating grain size.

Recent studies showed that some of the seed size regulators have conserved functions between rice and other plant species. For example, several components in BR signaling and G protein signaling influence seed size in both rice and *Arabidopsis* (Li and Li 2015, 2016); the ubiquitin ligase GW2 and its homologs in *Arabidopsis*, wheat and maize have conserved functions in seed size control (Bednarek et al. 2012; Li et al. 2010; Song et al. 2007; Xia et al. 2013). These pieces of evidences indicate that different plant species share similar mechanisms to control seed and organ growth. Therefore, studies on rice grain size could help us to understand the mechanisms of seed size control in other crops.

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