of the mitochondrial compartment, which contributes to the maintenance of functional mitochondria [11,12]. The MMF process also influences nucleoid maintenance. In protoplasts preparing for division, MMF results in all mitochondria having observable nucleoids [6], and it was suggested by Seguí-Simarro and Staehelin [9] that this process would have a similar effect in the shoot meristem and subsequent gamete formation, but in this example nucleoid distribution was not measured. Consistent with nucleoid maintenance as a consequence of MMF in the SAM, more than 90% of promitochondria in seed embryos were found to have observable nucleoids [7]. However, after MMF during germination only 67% of mitochondria have detectable nucleoids, thus re-establishing the heterogeneity of nucleoids seen in somatic cells [2–4,6]. Since nucleoid heterogeneity is largely overcome in mature cells by transient fusion and fission between mitochondrial pairs [2], a more important consequence of MMF therefore might be the exchange of proteins and maximizing mtDNA repair and recombination. Presumably at least some mitochondria ultimately fail to be 'renewed' by the MMF/fission processes and this could be resolved by autophagy. Paszkiewicz *et al.* [7] addressed this point and demonstrated upregulation of mitophagy early in the germination process.

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Spotlight

Boosting Rice Yield by Fine-Tuning *SPL* Gene Expression

Lei Wang[1] and Qifa Zhang[1,*]

Plant architecture is an important determinant of crop yield. Recent studies showed that *SPL* family genes regulate the architecture of rice plants. SPLs inhibit tillering in general, but promote panicle branching at optimal expression levels to increase grain number. Fine-tuning the expression of *SPL* genes may provide useful strategies for crop improvement.

Rice provides calories to half of the human population. At the individual plant level, rice grain yield is determined by three component traits: number of tillers (panicles), number of grains per panicle, and grain weight [1]. Breeding efforts using the semi-dwarf gene in the 1960s and development of hybrid rice in the 1970s–1980s brought about two great leaps in rice yield. In addition to the three component traits, it has long been realized that plant architecture, by shaping the population structure of the plants to maximize the holding capacity, is also a very important determinant of yield per unit area. Thus, in the Super Rice breeding programs in the 1990s, the International Rice Research Institute proposed the notion of a 'new plant type' (NPT), or ideotype, with the core concept of achieving a 'super high' yield by manipulating plant architecture to breed rice plants that bear fewer but effective tillers, bigger panicles, thick stems, medium height, erect leaves, and a vigorous root system [2]. Similar super-rice breeding programs were also launched in China, and these have achieved great success, especially in super-hybrid rice breeding. For future progress, it is crucial to understand the interrelationships between NPT characters, the genes controlling the traits, the mechanisms of their functions, and the regulatory networks.

Recently a paper published by a multi-institutional team, led by Zuhua He and Jiayang Li of the Chinese Academy of Sciences, revealed a new mechanism whereby the *IPA1* (*Ideal Plant Architecture 1*) gene regulates the traits that shape the architecture of the rice plant [3]. This work added fresh knowledge to the mechanistic understanding of plant architecture regulation, particularly in rice.

SPL Genes Play Major Roles in Plant Development

IPA1 encodes *Oryza sativa* (Os)SPL14, a member of the SQUAMOSA PROMOTER BINDING PROTEIN (SBP)-like (SPL) family of plant-specific transcription factors that all contain a highly conserved DNA-binding domain (SBP domain) of ~76 amino acids in length [4]. Since the identification of the first two members *AmSBP1* and *AmSBP2* in snapdragon

(*Antirrhinum majus*), *SPL* genes have been found in nearly all plant species including algae and moss [4].

It is now known that some *SPL* genes are regulated by microRNAs. MicroRNA156s (miR156s) and their mimics miR157s are highly conserved small RNA families in plants. Another small RNA, miR529, differing by four nucleotides from miR156/157, is also found in grasses and moss. Because of the high sequence similarity among these microRNAs, it is assumed that these three microRNA families target the same genes. Eleven of the 18 *SPL* genes in the rice genome contain sequences complementary to miR156/157/529 in their coding regions or 3′-untranslated regions, and thus are likely post-transcriptionally regulated by these small RNAs [4,5]. It has been demonstrated that the genetic module *miR156/157/529-SPL* controls a large range of processes underlying plant growth and development, including embryonic patterning, phase change, plant architecture, leaf development, flower structure, fruit maturation, tuberization, nodulation, immunity, secondary metabolism, and response to environmental stimuli [3–12].

SPL Genes Have Multiple Roles in Rice Yield

In 2010, two groups from China and Japan independently reported the function of *OsSPL14* identified by quantitative trait locus (QTL) cloning, and named the locus *IPA1* and *WFP* (*Wealthy Farmer's Panicle*), respectively. The variants of *OsSPL14* dampened tiller branching, but increased panicle branching and grain weight together with stronger culms [6,7]. Functional analysis showed that *IPA1* had a point mutation in the recognition site for miR156/157/529, while *WFP* changed epigenetic modification in the promoter region, both of which resulted in slightly elevated expression of *OsSPL14* (Figure 1A). These phenotypes were reminiscent of NPT characters, inspiring the idea of developing NPT rice by exploring *OsSPL14*. Effects of other *SPL* genes (e.g., *SPL7* and *SPL17*) on tillering and panicle branching were also observed in rice [5,8,9], indicating likely common functions of the *SPL* family genes in branching regulation. Moreover, the downstream targets of SPLs are also amenable for modification to produce changes in traits that affect architecture [5], providing additional targets for genetic manipulation.

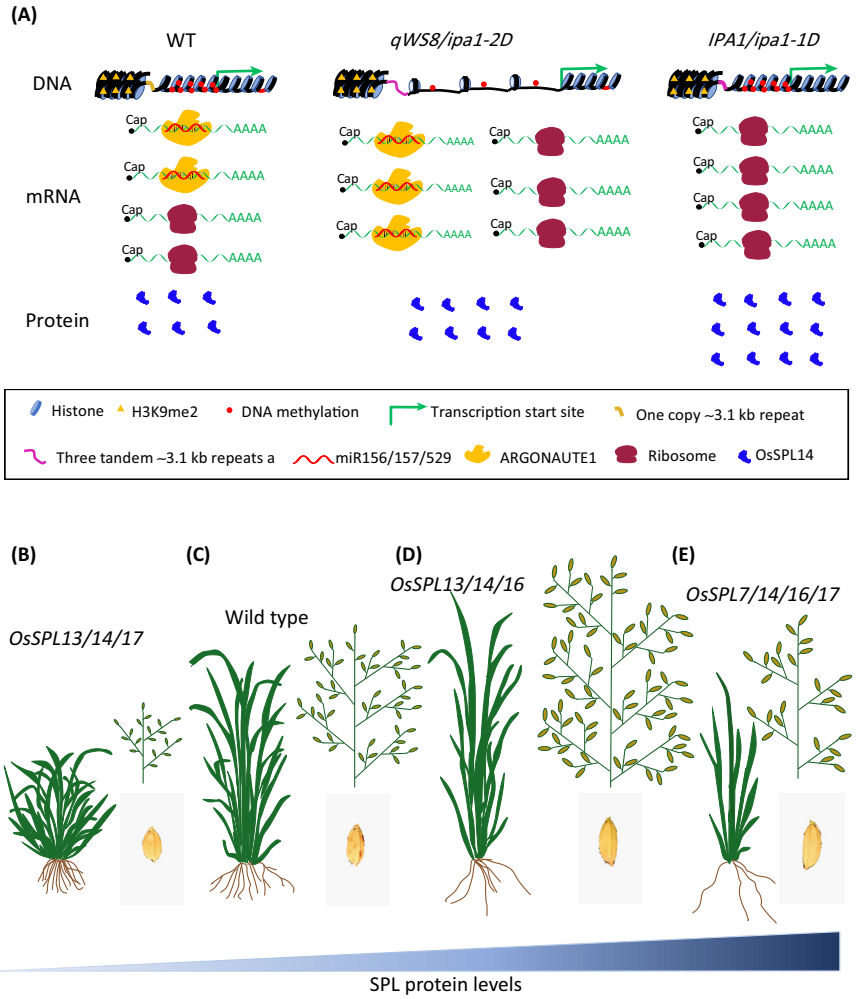

Figure 1. Regulating *SPL* Genes for Rice Yield Improvement. (A) Elevated *OsSPL14* expression by *IPA1/ipa1-1D* and *qWS8/ipa1-2D* through distinct mechanisms. In the wild type (WT), DNA methylation and heterochromatin (marked by histone H3 lysine 9 dimethylation, H3K9me2) occur in the upstream region of *OsSPL14*, resulting in relatively low transcription of the gene [3]. Most of the transcripts are degraded via miR156/157/529, leading to low level of the encoded protein. In the *qWS8/ipa1-2D* allele, three tandem 3.1 kb repeats in the upstream region of *OsSPL14* raise the transcriptional activity of *OsSPL14* by reducing DNA methylation and promoting an open chromatin state [3]. Because of the activity of miR156/157/529, the amounts of full-length transcript and the protein are only slightly increased compared to the wild type. In the *IPA1/ipa1-1D* allele, the change in the recognition site for miR156/157/529 greatly reduces post-transcriptional degradation, consequently both full-length transcript and the protein are substantially increased compared to the wild type and *qWS8/ipa1-2D* [3,6]. (B–E) Expression dosage effects of *SPL* genes on rice plant architecture and seed size. Plants with lower expression of *OsSPL13/14/17* (B) produce more tillers, but less panicle branching and smaller seeds, than the wild type (C). Plants with intermediate levels of *OsSPL13/14/16* (D) produce fewer tillers, but bigger panicles (more panicle branching) or bigger seeds; whereas plants with high levels of *OsSPL7/14/16/17* display greatly reduced tiller and panicle branching simultaneously (E). The bar at the bottom indicates SPL protein levels.

In this recent study, Zhang *et al*. identified another allele of *IPA1* (*qWS8/ipa1-2D*)

that also regulates the expression of *OsSPL14* [3]. However, the function of *qWS8/ipa1-2D* is exerted through three tandem repeats residing upstream of *OsSPL14*, compared to only one copy in the Nipponbare genome, which caused a reduction in the level of DNA methylation and a more open chromatin state in the promoter region, thus increasing the expression of *OsSPL14* (Figure 1A).

In addition to plant architecture, QTL analyses also showed that *SPL* genes regulate grain size, thus affecting grain yield in rice. For example, the expression levels of *OsSPL13* and *OsSPL16* modulate grain size, shape, and yield [8,9]. Grain size and shape are also important determinants of grain quality. Furthermore, the expression levels of *OsSPL13* and *OsSPL16* are also associated with tiller and panicle branching [8,9].

Taken together, all the identified QTLs that encode SPLs regulate rice yield by changing their expression patterns/levels rather than protein functions; slightly higher expression of the genes may result in favorable changes of the agronomic traits. Of note, all five *SPL* genes mentioned above, *OsSPL7*, *OsSPL13*, *OsSPL14*, *OsSPL16*, and *OsSPL17*, are likely targets of miR156/157/529. One possible explanation for their effects on tillering and panicle branching is that higher levels of *SPL* gene expression antagonize the roles of miR156/157/529, offsetting the negative effects of these microRNAs, as demonstrated using the microRNA target mimicry approach [5].

Exploring the Expression Dosage Effects of *SPL* Genes for Crop Improvement

Wang *et al*. proposed that fine-tuning *SPL* gene expression may provide a strategy for increasing rice productivity in breeding [5]. The available data from functional tests of *SPL* genes are in agreement with the idea of fine-tuning strategy (Figure 1B–E).

For example, compared to the previously reported *IPA1* allele (renamed *ipa1-1D*) [6], *ipa1-2D* is weaker in promoting *OsSPL14* expression. However, such weaker expression results in plants with an optimal combination of tiller number and panicle size, and thus increased grain yield [3]. This is realized by increased panicle branching and culm strength, with slight compensation of tiller numbers, compared to the heavy reduction of tiller branching by *IPA1/ipa1-1D*. Moreover, both tiller and panicle branching were greatly reduced by overexpressing *OsSPL7/14/16/17*, and at least the miR172-AP2 and *PAP2/OsMADS34–RCN1* pathways were involved in the reduction of panicle branching in these overexpression lines [5,8]. Thus, SPLs always inhibit tillering, but promote panicle branching only when at an optimal level. This conclusion is further strengthened by the fact that maize *UB3* (the ortholog of *OsSPL14*) promoted rice panicle branching in moderate overexpression lines, but the opposite was observed in high expression lines [10]. By contrast, in mutants or RNAi lines of all studied *SPL* genes, tillering was greatly enhanced while panicle branching was reduced (Figure 1B,C) [3–9]. Taken together, expression of *SPL* genes must be fine-tuned to favorable levels to increase productivity by balancing the pleiotropies (Figure 1B–E). Furthermore, it was recently reported that IPA1 interacts with IPI1 (IPA1 interacting protein 1) that encodes a RING-finger E3 ligase, thus promoting the degradation of IPA1 in panicles specifically, adding a mechanism for post-translational regulation to achieve the optimal level of IPA1 [11]. In addition to rice, *SPL* genes also control fruit ripening in tomato and kernel row number in maize in a dosage-dependent manner [4,12], suggesting that modifying the expression patterns/levels of *SPL* genes may provide a general strategy for crop genetic improvement.

Concluding Remarks

While the functions of other *SPL* genes still need to be tested, the findings so far have important implications in breeding applications in rice. The desirable alleles can be used directly to improve rice yield by traditional crossing approach, with or without marker-assisted selection. Gene-editing technology such as CRISPR/Cas9 can be used to modify the regulatory regions or miRNA recognition sites of *SPL* genes to modify their expression patterns/levels to create novel mutations for desirable tiller number and panicle size. Furthermore, dosage-dependent regulation by *SPL* genes should also be explored in other traits and other plants for crop genetic improvement.

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Spotlight

Autophagy: A Double-Edged Sword to Fight Plant Viruses

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In metazoans, autophagy is an essential component of host defense against viruses, orchestrating their degradation. Such antiviral functions for autophagy have also been long suspected in the green lineage. Two recent reports provide molecular insights on how plants selectively send viral proteins and even particles to the vacuole.

From the Greek meaning 'eating of self', autophagy is a process that leads to the degradation of intracellular material such as organelles, ribosomes, and protein aggregates, which becomes of particular importance under stress conditions such as starvation [1]. This intracellular material is engulfed into vesicles called autophagosomes that are subsequently delivered to lytic compartments: the lysosome in animal cells and the vacuole in yeast and plant cells. While autophagy works as a bulk catabolic process, it can also be highly selective thanks to a still-growing number of autophagy receptors that recruit the cargo substrate to the forming autophagosome by interaction with membrane-associated ATG8/LC3 proteins [2]. In humans autophagy malfunction has been linked to various diseases such as cancer and neurodegeneration, but it also plays a chief role in innate and adaptive immunity to virus infections. Hence, autophagy provides a potent antiviral mechanism by degrading viruses. Nevertheless, increasing evidence shows that viruses can manipulate this process to their own advantage [3,4], demonstrating the pivotal role of autophagy in the ongoing arms race between viruses and their hosts.

Autophagy-Dependent Degradation As an Antiviral Mechanism against Plant Viruses

Such interactions between viruses and autophagy are far less understood in plants, but previous microscopic observations of viral particles inside the vacuole have supported an active process for their clearance [5]. Moreover, autophagy is induced on viral infection in various plants and deficiency in autophagy can enhance virus accumulation. In addition it has been reported that the calmodulin-like protein rgsCaM from tobacco acts as a countermeasure to viral infection by binding to viral suppressors of RNA silencing (VSRs) such as the 2b protein from *Cucumber mosaic virus* (CMV) [6]. Strikingly, the impaired degradation of 2b on treatment with the autophagy inhibitor 3MA and silencing of beclin1/ATG6 strongly suggests that turnover of the VSR is achieved via the autophagy pathway. However, the way by which the autophagy machinery recruits the rgsCaM/2b complex is presently unknown.

Two recent reports provided novel molecular insights on the antiviral role of autophagy in compatible plant and DNA virus interactions. These molecular connections and others discussed in here are illustrated in Figure 1.

Haxim and colleagues [7] showed that autophagy helps to protect plants against three different geminiviruses (with circular single-stranded DNA genomes). Using the satellite-encoded protein βC1, a virulence factor of *Cotton leaf curl Multan virus* (CLCuMuV) to perform yeast two-hybrid screening of a tomato cDNA library, they identified an ATG8-related protein as an interactor. In autophagy, ATG8-related proteins are conjugated to phosphatidylethanolamine (PE) and required for autophagosome formation [1]. Notably, these proteins also interact with numerous proteins carrying an ATG8-family interacting motif (AIM), such as autophagy receptors, but also non-autophagy proteins, thereby enabling tethering of specific cargos to the autophagosome for vacuolar degradation.

βC1 interaction with NbATG8f (from *Nicotiana benthamiana*) was also observed and confocal microscopy revealed that the two proteins are colocalized and both are delivered to the vacuole in leaf cells. The authors further found that CLCuMuV infection induces autophagy and enhances autophagic flux. This suggests that autophagy may function as an antiviral mechanism against CLCuMuV by degrading βC1 via its recruitment to autophagosomes through ATG8-related proteins. To test this hypothesis, the authors undertook a genetic approach to modulate autophagy activity. Interestingly, infection by CLCuMuV of plants silenced for *NbATG5* and *NbATG7*, both required for autophagosome formation, increased leaf curl symptoms and viral DNA levels, while the converse was observed with activation of autophagy through downregulation of the cytosolic glyceraldehyde-3-phosphate dehydrogenase. Moreover, disrupting the interaction between βC1 and NbATG8f abrogated the autophagic antiviral defense against CLCuMuV. As βC1 has VSR activity, its autophagy-mediated degradation could also be beneficial to the virus by avoiding a too-severe infection, which might be deleterious for its host.

In another recent report, Hafrén and colleagues provide further support to this idea by revealing the complex interactions