

Review

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The convergence of autophagy, small RNA and the stress response – implications for transgenerational epigenetic inheritance in plants

Abstract: Recent discoveries in eukaryotes have shown that autophagy-mediated degradation of DICER and ARGONAUTE (AGO), the proteins involved in post-transcriptional gene silencing (PTGS), can occur in response to viral infection and starvation. In plants, a virally encoded protein P0 specifically interacts with AGO1 and enhances degradation through autophagy, resulting in suppression of gene silencing. In HeLa cells, DICER and AGO2 protein levels decreased after nutrient starvation or after treatment to increase autophagy. Environmental exposures to viral infection and starvation have also recently been shown to sometimes not only induce a stress response in the exposed plant but also in their unexposed progeny. These, and other cases of inherited stress response in plants are thought to be facilitated through transgenerational epigenetic inheritance, and the mechanism involves the PTGS and transcriptional gene silencing (TGS) pathways. These recent discoveries suggest that the environmentally-induced autophagic degradation of the PTGS and TGS components may have significant effects on inherited stress responses.

Keywords: autophagy; inherited stress response; post-transcriptional gene silencing; transcriptional gene silencing; transgenerational epigenetic inheritance.

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Introduction

Introduction to autophagy

Autophagy is process that involves proteins and other cytoplasmic components in the cell being delivered to a lysosome (in animals) or vacuole (in yeast and plants) for degradation. It is a survival mechanism that plays a crucial role in nutrient recycling, development, cell homeostasis, defence against pathogens and toxins (1). Autophagy consists of three stages: the phagophore, autophagosome, and autolysosome (animal) or autophagic body (plant) (Figure 1) (2). Each stage is regulated by autophagy-related (ATG) proteins. Much is known about the mechanism of autophagy in animal systems, but less is known about it in plants.

However, autophagy is a highly consistent mechanism in eukaryotic cells, and recent evidence has shown similar autophagic mechanisms in plants. For example, the *Arabidopsis* NBR1 protein, which is an ortholog of the mammalian autophagic cargo receptor, interacts with Atg8 ubiquitin-like protein through AIM (Atg8-interacting motif) for triggering selective autophagy (3). Furthermore, several *atg* mutants of *Arabidopsis* have shown accelerated leaf senescence and hypersensitivity to nutrition starvation, which was considered to trigger autophagy (1). Finally, *Atg* genes of *Arabidopsis* also have been shown to play important roles in biotic and abiotic stress responses and plant development (4–7), suggesting that autophagy crosstalks with many pathways.

Introduction to small RNAs in plants

MicroRNAs (miRNAs) and short-interfering RNAs (siRNAs) are small RNAs in animals and plants that have critical roles in development, epigenetic regulation, and pathogen defence (8). They are both 20–24 nucleotides (nt) long and function in either post-transcriptional gene silencing

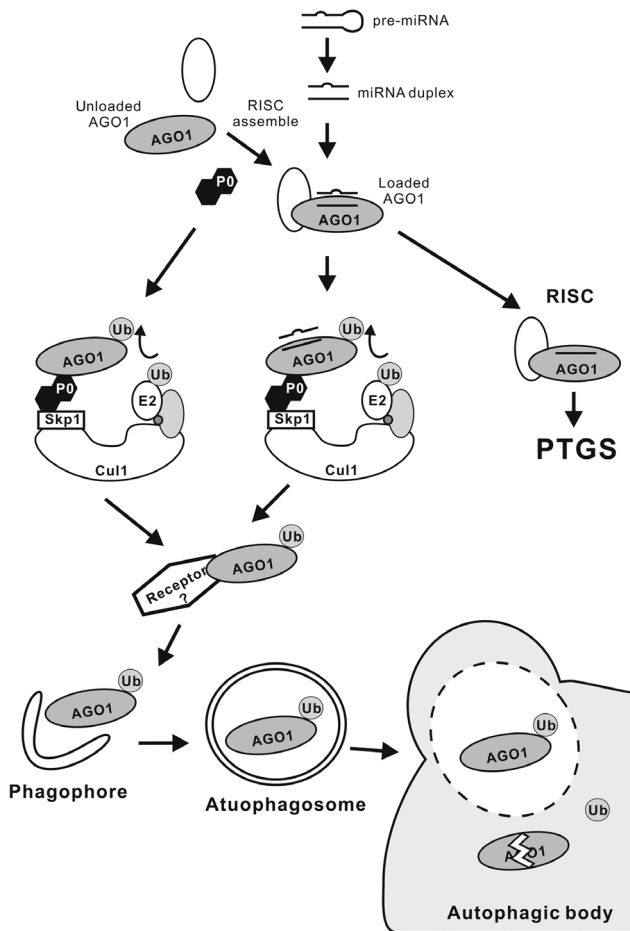


Figure 1 Model for P0-mediated autophagy for AGO1 degradation. The general miRNA pathway is a miRNA produced from miRNA precursor (pre-miRNA) to produce a miRNA duplex, and then assembled into an RNA-induced silencing complex (RISC) with AGO1 and other components. The miRNA* is erased during the process. The remaining miRNA forms a miRISC with AGO1 to determine the target mRNA. The P0 F-box-like domain binds unloaded or loaded AGO1 to the P0-SCF complex for ubiquitination. The ubiquitinated AGO1 might be recognised by the autophagy receptor and embed into the phagophore, and subsequently be transferred by the autophagosome. The outer membrane of the autophagosome merges with the membrane of the vacuole to form an autophagic body. AGO1 is finally degraded inside of the autophagic body.

(PTGS) or RNA-directed DNA methylation (RdDM). They differ in the type of RNA from which they are derived and in some of the proteins that produce them and that execute their functions. In general, both miRNAs and siRNAs perform PTGS through cleavage or translational inhibition of RNAs, while siRNAs can also facilitate transcriptional gene silencing (TGS) by directing epigenetic modifications to genomic or viral DNA (9, 10).

In mammalian cells, the Drosha-DGCR8 complex binds primary miRNA (pri-miRNA) and processes pri-miRNA to the precursor miRNA (pre-miRNA). Pre-miRNA

binds with Exportin-5-RanGTP complexes, which transfers the pre-miRNA from the nucleus to the cytoplasm (8). After Drosha-DGCR8 processing, DICER1 cleaves the pre-miRNA to generate a double-stranded form of miRNA duplex. In plants, Dicer-like 1 (DCL1) works with a double-stranded RNA-binding domain (dsRDB) – HYL1 – to generate a pre-miRNA and miRNA duplex in the nucleus (8). In animals and plants, the miRNA duplex is loaded into ARGONAUTE (AGO) and the complementary strand of miRNA (miRNA*) is erased, forming a RNA-induced-silencing complex (RISC) with a mature miRNA (miRISC) (8). Finally, the mature RISC goes on to cleave or translationally-inhibit mRNAs that have homology to the miRNA.

The siRNA pathway starts with a double-stranded RNA precursor that is processed by various DICERs in animals and plants (11). Endogenous (i.e., originating from the genome) siRNA, such as trans-acting siRNA (tasiRNA), plays an identical role to miRNA in downregulating endogenous mRNAs through PTGS (12, 13), while production of viral siRNAs are responsible for cleavage of viral genomic RNA in the plant as part of the virus defence response (14, 15). There are four Dicer-like proteins in *Arabidopsis thaliana* compared to the one in animals, and DCL2 and DCL4 have been reported to facilitate the viral defence response in a process called RNA-mediated resistance (16–18). However, siRNAs can also direct epigenetic change of the genome or viral DNAs. In plants, the siRNAs, which are generated by RNA-DEPENDENT RNA POLYMERASE 2 (RDR2), RNA polymerase IV (Pol IV), and Dicer-like 3 (DCL3), target homologous DNA sequences for epigenetic remodelling, which can result in RdDM (19, 20). Unlike PTGS, which uses ARGONAUTE1 (AGO1), the siRNAs are incorporated into ARGONAUTE 4 or 6 (AGO4, or AGO6) in the RdDM pathway. AGO4 and the other components of the RdDM complex can reprogram DNA methylation, histone modifications and higher order chromatin state, resulting in TGS (21–23).

Inherited stress responses in plants

As plants are unable to move to new locations they have evolved rapid adaptive biological systems to deal with stressful changes in their local environment. These environmental changes can be grouped into biotic (e.g., pathogen infection, herbivory) and abiotic (e.g., temperature, nutrient availability, toxin levels, drought). Each stress elicits a response that can alter the gene expression program at a contained location or throughout the whole plant and ultimately leads to phenotypic change. The phenotypic changes aim to physiologically counter the stress or can even lead to a developmental change that usually

involves an earlier progression to flowering and seed dispersal than unstressed plants (24). A large amount of studies have characterised the molecular changes in various stress responses in plants, including hundreds of alterations to small RNAs (miRNAs and siRNAs) (25). Changes to both global and locus-specific epigenetic states have also been described in various stress responses (26).

However, several studies have indicated that the progenies from stressed parental plants have inherited the molecular responses to that stress (26). This is presumed to be an adaptive mechanism that allows the next generation to be prepared for the environment in which they will live. Examples of this transgenerational memory have been shown with plants that are exposed to a wide range of stresses, both abiotic and biotic (26, 27). The precise mechanisms of the inherited responses are not understood but it is thought that the transfer of stress-induced RNAs, metabolites, hormones or epigenetic modifications in the gametes is involved. It is important to note that not all stress responses are inherited so there must also be some mechanisms through which they are reset between generations. Furthermore, some prominent researchers have put forward stringent criteria that they suggest are required to ensure that there is unambiguous evidence for transgenerationally-inherited stress responses (28).

In general, the offspring of stressed plants have often been found to have increased global DNA methylation as well as localised hypomethylation (26). DNA methylation at CG dinucleotides is highly stable in plants and reduction in global CG methylation caused by mutations in the enzymes responsible for its maintenance, MET1 and DDM1 can persist for more than five generations (29, 30) (Box 1). In spite of this making the CG methylation systems apparently ideal for facilitating transgenerational epigenetic inheritance in plants, it is the more dynamic non-CG (CHG

and CHH sites, where H is A, T, or C) DNA methylation systems that have been connected with inherited stress responses.

In plants, the RdDM pathway plays an important role in setting non-CG methylation on genomic DNA. 24-nt siRNA biogenesis depends on Pol IV, RDR2, and DCL2-4. Downstream of the siRNA-production, other RdDM components, including the DNA methyltransferase DRM2, AGO4, and Pol V, are associated with the siRNAs to target the genomic loci, which can cause TGS (21). Deletion of components of the RdDM pathway have been shown to reduce or prevent inherited stress responses to herbivory, drought, heat, cold and ultra violet C radiation (31, 32). However, it is currently not clear whether it is the small RNAs themselves, the siRNA-directed epigenetic modifications – or both – that create the transgenerational memory. Clues perhaps lie in the recent description of RdDM that occurs in pollen, egg cells and early embryos in *Arabidopsis* that serves to establish repeat-element silencing as well as methylation at alleles that display transgenerationally-stable epigenetic states (epialleles) (33, 34).

Finally it should be noted that there is overlap in the CG, and non-CG methylation mechanisms as small RNAs were shown to guide CG remethylation of repeat elements in MET1 null mutant plants (35). Therefore, future research may reveal a role for CG methylation in the inherited stress responses.

DICER and AGO levels are regulated through autophagy

A variety of recent research has revealed a mechanism for targeted degradation of components of the PTGS system

Box 1 Epigenetic inheritance

Methylation of cytosines (DNA methylation) and post-translational modification of histones are epigenetic ‘marks’ that can persist at a region of the genome for many cell divisions and even sometimes between generations. There are multiple mechanisms that facilitate the replication of epigenetic marks, either in daughter cells or in offspring [reviewed in (19), Law and Jacobsen (2010)]. DNA methylation can be duplicated at replication forks by maintenance methyltransferases that methylate cytosines on the nascent strand using the existing methylation on the template strand as a guide. DNA methylation patterns can also be maintained by repeated *de novo* methylation, often with RdDM mechanisms. Removal of DNA methylation can occur passively by not replicating it in newly synthesised DNA, or actively through enzymatic mechanisms.

Less is known about how the histones that are bound to the newly formed DNA are modified to recreate the epigenetic patterns that were present before replication [reviewed in (52), Zhu and Reinberg (2011)]. Indeed, different mechanisms may exist for different types of modification, or even for the same modification but in different circumstances, e.g., transcriptional regulation vs. chromosome structural maintenance roles. The mechanism that is currently most favoured for several modification-types involves the sharing of the modified nucleosomes that were in the parental chromatin between the two nascent daughter chromosomes. Subsequently those inherited nucleosomes attract epigenetic remodelling complexes that place the same modifications on the neighbouring ‘new’ unmodified nucleosomes.

through autophagy. This process is part of PTGS system homeostasis but can also be triggered by environmental stresses such as viral infection and nutrient deprivation. Gibbings et al. (2012) (36) demonstrated that the DICER and AGO2 protein levels of HeLa cells decreased after nutrient starvation or when treated with rapamycin to induce autophagy, whereas increased DICER and AGO2 protein levels were observed after treatment with lysosomal inhibitors bafilomycin A1 (BAF) or chloroquine (CQ) to block autophagy. Martinez et al. (2013) (37) reported that the ubiquitination inhibitor MG132 did not suppress the degradation of AGO2 in mouse embryonic stem cells (ESCs), which indicates that proteosomal degradation was not the AGO2-reducing mechanism. However, treatment with different autophagy inhibitors in ESCs showed stabilisation of the AGO2 protein levels. Furthermore, AGO2 associates with multivesicular bodies for secretion and lysosomal degradation in ESCs (37). These findings indicate core components of PTGS are regulated or fine-tuned by autophagy-mediated degradation (36, 37). Derrien and colleagues also demonstrated that plant AGO1 is degraded by autophagy (38), and we later describe this process in-depth.

Mechanism of selective autophagic degradation of core components of PTGS

The autophagy receptors (p62, and NDP52) bind with substrates and ATG8 family proteins, and integrate the complexes into the membrane of the autophagosome for selective degradation (39, 40). Gibbings et al. (2012) (36) showed that the autophagy receptor NDP52 was responsible for targeting unloaded AGO2 for degradation. Moreover, DICER significantly co-localised with autophagy receptor NDP52 in HeLa cells. The co-localisation foci increased 3.2-fold in cells treated with RAP, suggesting that NDP52-dependent autophagy targets DICER. Moreover, bioimage evidence showed that DICER and AGO2 were enriched in autophagosomes and autolysosomes of HeLa cells when treated with CQ inhibitor, indicating that DICER and AGO2 are subjected to NDP52-dependent autophagic degradation (36).

MiRNA loading in RISC determines AGO stability

The interactions between small RNAs and their associated proteins are not only required for small RNA biosynthesis and function. Recent work has shown that the

interactions also coordinate the turn-over of the proteins themselves. DGCR8 null mouse ESCs lacked mature miRNA accumulation and displayed significant reduction of AGO2 protein levels compared with wild-type ESCs (37). However, the AGO2 protein levels increased when DGCR8 null ESCs were complemented with Flag-DGCR8 (37). In addition, introduction of miRNA precursor or siRNA duplexes in the ESCs resulted in an increase of AGO2 protein levels, indicating that the AGO2 stability depended on the loaded miRNAs (37). Finally, this study also implicates autophagy in the reduction of AGO2 as its degradation was blocked by inhibition of the lysosome, but not of the proteasome. Therefore, while the protein machinery of small RNA biogenesis will determine the amount of small RNAs in a cell, the opposite can also occur, i.e. the level of small RNAs can determine the level of proteins. This self-regulating system is thought to achieve a homeostasis of the system but also there is evidence that DICER-ARGONAUTE complexes that do not carry small RNAs can actually interfere with the normal function of those that do (36, 41, 42).

Viral suppressor triggers autophagic AGO1 degradation in plants

In plants, PTGS is commonly used as a virus defence system. To counter this, viruses have evolved methods to suppress the RNA-mediated resistance, resulting in gene silencing suppression of the miRNA and siRNA pathways (43, 44). Most viral suppressors have the ability to bind to miRNA and siRNA duplexes, preventing the small RNA duplexes from loading into the AGO proteins, such as p19, 2b, p21, and HC-Pro (45, 46). P0 is a viral suppressor of *Polevirus* that contains an F-box-like motif, and hijacks the host S-phase kinase-associated protein 1 (SKP1) and cullin 1 (CUL1) to form the SCF complex, which is involved in ubiquitination (Figure 1) (47). In addition, P0 has been reported to interact with AGO1 of plants (the major ribonuclease III for miRNA- and siRNA-mediated cleavage) and trigger AGO1 degradation (38, 48). Pazhouhandeh et al. showed that mutated P0 F-box motif inhibits gene silencing suppression (47). However, P0-mediated AGO1 degradation was not affected by treatment with ubiquitination inhibitor MG132 in an experiment that was similar to the mouse cell-line work of Martinez et al. (37), but suppressed by MLN-4924, an inhibitor of neddylation, which is an analogous process to ubiquitination (38, 49). These results imply that inhibition of CUL1 neddylation resulted in impaired AGO1 degradation (38). Furthermore, Derrien et al. (2012) demonstrated that cysteine protease inhibitor

E-64d, blocks the activity of lysosomal hydrolases, treatment stabilises AGO1 protein levels in the presence of P0 in the cell, indicating that P0 triggers AGO1 degradation by the autophagic pathway. The destabilisation of AGO1 involves neddylation and autophagy but not ubiquitination-proteasome degradation.

The virally encoded suppressor protein P0 contains an F-box-like domain to hijack SKP1 and CUL1 to form an SCF complex and interact with AGO1 (Figure 1) (48, 49). The P0-SKP1 interaction is required for virus pathogenicity and depletion of SKP1 resulted in host-resistance to *Polerovirus* (47). Mutations in the F-box-like domain of P0 caused less progeny of viral RNA, with milder symptoms in the host plant than in the wild-type virus, and also inhibited suppression of PTGS (47). P0 requires the ND and the PAZ protein domains of AGO1 for AGO1 interaction and destabilisation (48). However, P0 does not affect Dicer-like 1 (DCL1; DICER homolog) stabilisation, although the PAZ domain also exists in DCL1, suggesting that the ND domain plays an adjacent role with the PAZ domain in P0 recognition and regulation (48).

Bioimaging has provided more information on the AGO1 autophagy mechanism. This technique showed that AGO1 co-localises with ATG genes in vesicles (38). ATG8a covalently attaches to the lipid phosphatidylethanolamine (PE) to bind to autophagosomal membranes by means of its lipid moiety. Co-expression of GFP-AGO1 and red fluorescence protein (RFP)-ATG8a fusion proteins showed that AGO1 and ATG8a co-localised in autophagic vesicles. The vesicles become larger when treated with E-64d (38). In addition, ATG8a co-immunoprecipitated with AGO1 in E-64d-treated *Arabidopsis* plants, indicating that AGO1 interacts with ATG8a (38).

Implications of DICER and AGO degradation for the inherited stress response

The recent research outlined above indicates that components of the PTGS system can be specifically targeted for autophagy. We propose that targeted degradation of the PTGS or TGS systems may influence inherited stress responses and suggest that further research on this possibility is warranted. Changes to small RNA systems could potentially either inhibit or facilitate mechanisms of inherited stress responses. The destabilisation of AGO1 by viruses clearly serves to enable the virus to elude the small RNA-mediated defence systems. However, the reduction in

AGO1 by viruses may not only reduce the plant's ability to cleave viral RNAs or methylate viral genomes, but it could also reduce small RNA-mediated transgenerational inheritance mechanisms. The virus would therefore be making the infected plant's offspring more easily infected.

No direct evidence exists to prove that autophagy in plants regulates the components of RdDM. However, AGO4 and AGO6 are homologs of AGO1, so it is possible that autophagy could also target RdDM components, and through that could also alter small RNA-mediated transgenerational memory (either siRNAs, miRNAs or DNA methylation). Indeed, the viral suppressor 2b of Cucumber mosaic virus (CMV) has been demonstrated to directly interact with AGO4 and to mediate transgene hypomethylation (50). CMV 2b also has small RNA-binding activity, implying that it might cause unloading of AGO4, resulting in suppression of RdDM. Therefore, future work should aim to uncover what components of the PTGS and TGS systems are specifically targeted for autophagy.

As well as the possibility that the RdDM machinery can be specifically targeted for autophagy, it is likely that the machinery will be affected by the general increases in the levels of autophagy that have been well documented as part of integrated stress responses to reactive oxygen species, nutrition starvation, high salt, drought and viral infection (51). So far the only stress other than viral infection that has been shown to induce autophagy of small RNA systems is nutrient starvation (37). It will be interesting to learn if other environmental stresses destabilise the PTGS and TGS systems.

Substantial shifts in the ability of a plant to perform PTGS or TGS would be expected to impact the transmission of small RNAs or RNA-directed epigenetic modifications from that plant. However, this needs to be tested in models of inherited stress response. Low nutrient-induced inherited DNA methylation changes have been described in dandelions (51). It would be interesting to alter the autophagy systems in this and similar models to see whether the inherited stress response and/or inherited DNA methylation patterns were affected.

It is unclear whether autophagy of components of inherited stress responses systems would be adaptive to help a plant prepare its offspring for a certain environment, or whether it would be an unintended consequence of measures that the parental generation take to counter a stress. For example, a reduction in small RNAs that direct epigenetic change because of destabilisation of AGO proteins could lead to hypomethylation of stress response genes in the gametes and a heightened stress response in the offspring. Alternatively, the same reduction in efficiency of the small RNA pathways could lessen the amount

of siRNAs in the gametes and thus impair the inherited stress response. The growing realisation of the importance of autophagy and non-genetic inheritance in plants, combined with how little is known about how these processes are intertwined, and the convergence of their mechanistic

components, suggests that more research should be done in this area.

Received October 6, 2013; accepted November 22, 2013

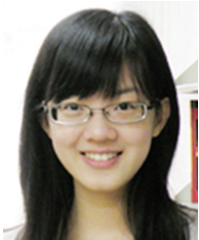
References

- Kim SH, Kwon C, Lee JH, Chung T. Genes for plant autophagy: functions and interactions. *Mol Cells* 2012; 34: 413–23.
- Klionsky DJ, Abdalla FC, Abeliovich H, Abraham RT, Acevedo-Arozena A, Adeli K, et al. Guidelines for the use and interpretation of assays for monitoring autophagy. *Autophagy* 2012; 8: 445–544.
- Svenning S, Lamark T, Krause K, Johansen T. Plant NBR1 is a selective autophagy substrate and a functional hybrid of the mammalian autophagic adapters NBR1 and p62/SQSTM1. *Autophagy* 2011; 7: 993–1010.
- Lenz HD, Haller E, Melzer E, Kober K, Wurster K, Stahl M, Bassham DC, Vierstra RD, Parker JE, Bautor J, Molina A, Escudero V, Shindo T, van der Hoorn RA, Gust AA, Nürnberger T. Autophagy differentially controls plant basal immunity to biotrophic and necrotrophic pathogens. *Plant J* 2011; 66: 818–30.
- Yoshimoto K, Hanaoka H, Sato S, Kato T, Tabata S, Noda T, Ohsumi Y. Processing of ATG8s, ubiquitin-like proteins, and their deconjugation by ATG4s are essential for plant autophagy. *Plant Cell* 2004; 16: 2967–83.
- Liu Y, Xiong Y, Bassham DC. Autophagy is required for tolerance of drought and salt stress in plants. *Autophagy* 2009; 5: 954–63.
- Shin JH, Yoshimoto K, Ohsumi Y, Jeon JS, An G. OsATG10b, an autophagosome component, is needed for cell survival against oxidative stresses in rice. *Mol Cells* 2009; 27: 67–74.
- Kim VN. MicroRNA biogenesis: coordinated cropping and dicing. *Nat Rev Mol Cell Biol* 2005; 6: 376–85.
- Vaucheret H, Fagard M. Transcriptional gene silencing in plants: targets, inducers and regulators. *Trends Genet* 2001; 17: 29–5.
- Fagard M, Vaucheret H. Systemic silencing signal(s). *Plant Mol Biol* 2000; 43: 285–93.
- Vaucheret H. Plant ARGONAUTES. *Trends Plant Sci* 2008; 13: 350–8.
- Allen E, Xie Z, Gustafson AM, Carrington JC. microRNA-directed phasing during trans-acting siRNA biogenesis in plants. *Cell* 2005; 121: 207–21.
- Vazquez F, Vaucheret H, Rajagopalan R, Lepers C, Gasciolli V, Mallory AC, Hilbert JL, Bartel DP, Crété P. Endogenous trans-acting siRNAs regulate the accumulation of *Arabidopsis* mRNAs. *Mol Cell* 2004; 16: 69–79.
- Jan FJ, Fagoaga C, Pang SZ, Gonsalves D. A minimum length of N gene sequence in transgenic plants is required for RNA-mediated tospovirus resistance. *J Gen Virol* 2000; 81: 235–42.
- Prins M. Broad virus resistance in transgenic plants. *Trends Biotechnol* 2003; 21: 373–5.
- Bouché N, Laussergues D, Gasciolli V, Vaucheret H. An antagonistic function for *Arabidopsis* DCL2 in development and a new function for DCL4 in generating viral siRNAs. *EMBO J* 2006; 25: 3347–56.
- Deleris A, Gallego-Bartolome J, Bao J, Kasschau KD, Carrington JC, Voinnet O. Hierarchical action and inhibition of plant Dicer-like proteins in antiviral defense. *Science* 2006; 313: 68–71.
- Wang XB, Jovel J, Udornporn P, Wang Y, Wu Q, Li WX, Gasciolli V, Vaucheret H, Ding SW. The 21-nucleotide, but not 22-nucleotide, viral secondary small interfering RNAs direct potent antiviral defense by two cooperative argonautes in *Arabidopsis thaliana*. *Plant Cell* 2011; 23: 1625–38.
- Law JA, Jacobsen SE. Establishing, maintaining and modifying DNA methylation patterns in plants and animals. *Nat Rev Genet* 2010; 11: 204–20.
- Wassenegger M, Heimes S, Riedel L, Sängler HL. RNA-directed *de novo* methylation of genomic sequences in plants. *Cell* 1994; 76: 567–76.
- Huetzel B, Kanno T, Daxinger L, Bucher E, van der Winden J, Matzke AJ, Matzke M. RNA-directed DNA methylation mediated by DRD1 and Pol IVb: a versatile pathway for transcriptional gene silencing in plants. *Biochim Biophys Acta* 2007; 1769: 358–74.
- Zheng X, Zhu J, Kapoor A, Zhu JK. Role of *Arabidopsis* AGO6 in siRNA accumulation, DNA methylation and transcriptional gene silencing. *EMBO J* 2007; 26: 1691–701.
- Feng S, Jacobsen SE. Epigenetic modifications in plants: an evolutionary perspective. *Curr Opin Plant Biol* 2011; 14: 179–86.
- Yaish MW, Colasanti J, Rothstein SJ. The role of epigenetic processes in controlling flowering time in plants exposed to stress. *J Exp Bot* 2011; 62: 3727–535.
- Khraiweh B, Zhu JK, Zhu J. Role of miRNAs and siRNAs in biotic and abiotic stress responses of plants. *Biochim Biophys Acta* 2012; 1819: 137–48.
- Migicovsky Z, Kovalchuk I. Changes to DNA methylation and homologous recombination frequency in the progeny of stressed plants. *Biochem Cell Biol* 2013; 91: 1–5.
- Paszowski J, Grossniklaus U. Selected aspects of transgenerational epigenetic inheritance and resetting in plants. *Curr Opin Plant Biol* 2011; 14: 195–203.
- Pecinka A, Mittelsten Scheid O. Stress-induced chromatin changes: a critical view on their heritability. *Plant Cell Physiol* 2012; 53: 801–8.
- Kakutani T, Munakata K, Richards EJ, Hirochika H. Meiotically and mitotically stable inheritance of DNA hypomethylation induced by *ddm1* mutation of *Arabidopsis thaliana*. *Genetics* 1999; 151: 831–8.
- Mathieu O, Reinders J, Caikovski M, Smathajitt C, Paszowski J. Transgenerational stability of the *Arabidopsis* epigenome is coordinated by CG methylation. *Cell* 2007; 130: 851–62.

31. Boyko A, Kovalchuk I. Transgenerational response to stress in *Arabidopsis thaliana*. *Plant Singal Behav.* 2010; 5: 995–8.
32. Rasmann S, De Vos M, Casteel CL, Tian D, Halitschke R, Sun JY, Agrawal AA, Felton GW, Jander G. Herbivory in the previous generation primes plants for enhanced insect resistance. *Plant Physiol* 2012; 158: 854–63.
33. Calarco JP, Borges F, Donoghue MT, Van Ex F, Jullien PE, Lopes T, Gardner R, Berger F, Feijó JA, Becker JD, Martienssen RA. Reprogramming of DNA methylation in pollen guides epigenetic inheritance via small RNA. *Cell* 2012; 151: 194–205.
34. Jullien PE, Susaki D, Yelagandula R, Higashiyama T, Berger F. DNA methylation dynamics during sexual reproduction in *Arabidopsis thaliana*. *Curr Biol* 2012; 22: 1825–30.
35. Teixeira FK, Heredia F, Sarazin A, Roudier F, Boccara M, Ciaudo C, Cruaud C, Poulain J, Berdasco M, Fraga MF, Voinnet O, Wincker P, Esteller M, Colot V. A role for RNAi in the selective correction of DNA methylation defects. *Science* 2009; 323: 1600–4.
36. Gibbings D, Mostowy S, Jay F, Schwab Y, Cossart P, Voinnet O. Selective autophagy degrades DICER and AGO2 and regulates miRNA activity. *Nat Cell Biol* 2012; 14: 1314–21.
37. Martinez NJ, Gregory RI. Argonaute2 expression is post-transcriptionally coupled to microRNA abundance. *RNA* 2013; 19: 605–12.
38. Derrien B, Baumberger N, Schepetilnikov M, Viotti C, De Cillia J, Ziegler-Graff V, Isono E, Schumacher K, Genschik P. Degradation of the antiviral component ARGONAUTE1 by the autophagy pathway. *Proc Natl Acad Sci USA* 2012; 109: 15942–6.
39. Pankiv S, Clausen TH, Lamark T, Brech A, Bruun JA, Outzen H, Øvervatn A, Bjørkøy G, Johansen T. p62/SQSTM1 binds directly to Atg8/LC3 to facilitate degradation of ubiquitinated protein aggregates by autophagy. *J Biol Chem* 2007; 282: 24131–45.
40. Thurston TL, Wandel MP, von Muhlinen N, Foeglein A, Randow F. Galectin 8 targets damaged vesicles for autophagy to defend cells against bacterial invasion. *Nature* 2012; 482: 414–8.
41. Chendrimada TP, Gregory RI, Kumaraswamy E, Norman J, Cooch N, Nishikura K, Shiekhattar R. TRBP recruits the Dicer complex to Ago2 for microRNA processing and gene silencing. *Nature* 2005; 436: 740–4.
42. Sakurai K, Amarzguioui M, Kim DH, Alluin J, Heale B, Song MS, Gatignol A, Behlke MA, Rossi JJ. A role for human Dicer in pre-RISC loading of siRNAs. *Nucleic Acids Res* 2011; 39: 1510–25.
43. Li WX, Ding SW. Viral suppressors of RNA silencing. *Curr Opin Biotechnol* 2001; 12: 150–4.
44. Wu HW, Lin SS, Chen KC, Yeh SD, Chua NH. Discriminating mutations of HC-Pro of zucchini yellow mosaic virus with differential effects on small RNA pathways involved in viral pathogenicity and symptom development. *Mol Plant Microbe Interact* 2010; 23: 17–28.
45. González I, Rakitina D, Semashko M, Taliensky M, Praveen S, Palukaitis P, Carr JP, Kalinina N, Canto T. RNA binding is more critical to the suppression of silencing function of Cucumber mosaic virus 2b protein than nuclear localization. *RNA* 2012; 18: 771–82.
46. Lakatos L, Csorba T, Pantaleo V, Chapman EJ, Carrington JC, Liu YP, Dolja VV, Calvino LF, López-Moya JJ, Burgyán J. Small RNA binding is a common strategy to suppress RNA silencing by several viral suppressors. *EMBO J* 2006; 25: 2768–80.
47. Pazhouhandeh M, Dieterle M, Marrocco K, Lechner E, Berry B, Brault V, Hemmer O, Kretsch T, Richards KE, Genschik P, Ziegler-Graff V. F-box-like domain in the polerovirus protein P0 is required for silencing suppressor function. *Proc Natl Acad Sci USA* 2006; 103: 1994–9.
48. Bortolamiol D, Pazhouhandeh M, Marrocco K, Genschik P, Ziegler-Graff V. The Polerovirus F box protein P0 targets ARGONAUTE1 to suppress RNA silencing. *Curr Biol* 2007; 17: 1615–21.
49. Baumberger N, Tsai CH, Lie M, Havecker E, Baulcombe D. The Polerovirus silencing suppressor P0 targets ARGONAUTE proteins for degradation. *Curr Biol* 2007; 17: 1609–14.
50. Hamera S, Song X, Su L, Chen X, Fang R. Cucumber mosaic virus suppressor 2b binds to AGO4-related small RNAs and impairs AGO4 activities. *Plant J* 2012; 69: 104–15.
51. Verhoeven KJ, Jansen JJ, van Dijk PJ, Biere A. Stress-induced DNA methylation changes and their heritability in asexual dandelions. *New Phytol* 2010; 185: 1108–18.
52. Zhu B, Reinberg D. Epigenetic inheritance: uncontested? *Cell Res* 2011; 21: 435–41.



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