integration events. The choice of tissue to be transformed was dictated by the need to use material that could be used for both transformation by *Agrobacterium* and for regeneration. This was achieved using small, vigorously dividing calli derived from mature seeds of rice. Indeed, optimization for *Agrobacterium*-mediated transformation was probably crucial to the success of GT in rice. Finally, an important factor that might have contributed to the relatively high GT frequency was the transcriptional activity of the targeted gene in the callus culture (S. Iida and K. Shimamoto, pers. commun.). Hopefully, this targeting strategy will also be used successfully in other endogenous rice genes and other important crop plants.

Acknowledgements

We acknowledge discussions with Shigeru Iida and Ko Shimamoto. We are grateful to Pawel Pelczar for improving the manuscript. We acknowledge financial support by the Novartis Research Foundation, and the Deutsche Forschungs-Gemeinschaft, respectively. We are also supported by the EU grant for the project 'Recombio'.

References

1 Isshiki, M. *et al.* (1998) A naturally occurring functional allele of the rice waxy locus has a GT to TT mutation at the 5′ splice site of the first intron. *Plant J.* 15, 133–138
2 Terada, R. *et al.* (2002) Efficient gene targeting by homologous recombination in rice. *Nat. Biotechnol.* 20, 1030–1034
3 Puchta, H. and Hohn, B. (1996) From centimorgans to basepairs: homologous recombination in plants. *Trends Plant Sci.* 1, 340–348
4 Rong, Y.S. (2002) Targeted mutagenesis by homologous recombination in *D. melanogaster*. *Genes Dev.* 16, 1568–1581
5 Schaefer, D.G. (2002) A new moss genetics: targeted mutagenesis in *Physcomitrella patens*. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 53, 477–501
6 Paszkowski, J. *et al.* (1988) Gene targeting in plants. *EMBO J.* 7, 4021–4026
7 Kempin, S.A. *et al.* (1997) Targeted disruption in *Arabidopsis*. *Nature* 389, 802–803
8 Hanin, M. *et al.* (2001) Gene targeting in *Arabidopsis*. *Plant J.* 28, 671–677
9 Reiss, B. *et al.* (2000) RecA stimulates sister chromatid exchange and the fidelity of double-strand break repair, but not gene targeting, in plants transformed by *Agrobacterium*. *Proc. Natl. Acad. Sci. U. S. A.* 97, 3358–3363
10 Shalev, G. *et al.* (1999) Stimulation of homologous recombination in plants by expression of the bacterial resolvase RuvC. *Proc. Natl. Acad. Sci. U. S. A.* 96, 7398–7402
11 Gorbunova, V. *et al.* (2000) A new hyperrecombinogenic mutant of *Nicotiana tabacum*. *Plant J.* 24, 601–611
12 Gherbi, H. *et al.* (2001) Homologous recombination in planta is stimulated in the absence of Rad50. *EMBO Rep.* 2, 287–291
13 Puchta, H. (2002) Gene replacement by homologous recombination in plants. *Plant Mol. Biol.* 48, 173–182
14 Goff, S.A. *et al.* (2002) A draft sequence of the rice genome (*Oryza sativa* L. ssp. *japonica*). *Science* 296, 92–100
15 Yu, J. *et al.* (2002) A draft sequence of the rice genome (*Oryza sativa* L. ssp. *indica*). *Science* 296, 79–92
16 Offringa, R. *et al.* (1993) Nonreciprocal homologous recombination between Agrobacterium transferred DNA and a plant chromosomal locus. *Proc. Natl. Acad. Sci. U. S. A.* 90, 7346–7350
17 Rubin, E. and Levy, A.A. (1997) Abortive gap repair: the underlying mechanism for Ds elements formation. *Mol. Cell. Biol.* 17, 6294–6302
18 Puchta, H. (1998) Repair of genomic double-strand breaks in somatic plant cells by one-sided invasion of homologous sequences. *Plant J.* 13, 331–339

1360-1385/03/$ - see front matter
doi:10.1016/S1360-1385(03)00004-9

DNA–RNA–protein gang together in silence

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Two recent reports demonstrate interdependence between DNA and histone methylation in *Arabidopsis*. *ddm1* (*decrease in DNA methylation 1*) mutants switch histone methylation from a form associated with inactive chromatin to a form connected to actively transcribed genomic regions. The loss of DNA methylation and shift in histone methylation cause transcriptional derepression of heterochromatic regions. In a related report, small RNAs in *Schizosaccharomyces pombe* mark histone methylation to form heterochromatin, suggesting that methylation systems work alongside RNA metabolism.

Gene regulation can be modeled after an old-fashioned telephone switchboard, where a multitude of switches regulates lines of communication. Likewise, various epigenetic switches are required for genomes to regulate their gene activity. Since the 1970s, DNA methylation has been recognized as an important epigenetic mark that generally correlates positively with transcriptional gene silencing. However, recent research has shown that another type of methylation is also important for gene regulation: histone methylation. Further, research supports the idea that both types of epigenetic marking systems interconnect in ways that are only beginning to be understood. Now, Anne-Valérie Gendrel and colleagues [1] and Lianna Johnson and colleagues [2] highlight the connection between the two systems of methylation by showing that reductions in DNA methylation reduce histone methylation in portions of the genome that are either heterochromatic or susceptible to epigenetic alterations.

Two methylation systems connect

Post-translational modification of histone tails modifies chromatin structure and subsequently affects gene expression. Methylated H3 histones come in at least two types, with methylation on lysine 4 (H3mK4) associated with active chromatin, and methylated lysine 9 (H3mK9)

associated with inactive chromatin [3]. Both groups used chromatin immunoprecipitation (ChIP), an important molecular tool used to monitor the status of H3 histone methylation on specific portions of the genome, and focused on *Arabidopsis*, which has available mutants in DNA and histone methylation.

The surprising result is that loss of DNA methylation affects the patterns of histone methylation in heterochromatic portions of the genome and results in transcriptional derepression. Both groups tested *decrease in DNA methylation 1* (*ddm1*) mutants that have an overall reduction of 70% of cytosine methylation. Gendrel and colleagues found that although the *ddm1* DNA methylation-deficient background does not cause overall reductions in histone methylation, it does cause a switch from H3mK9 to H3mK4 in a heterochromatic region that the group tested. The region also becomes derepressed and both hypothetical genes and transposable elements become transcriptionally active. Gendrel *et al*. argue that histone methylation correlates with alterations in gene expression, although the group did not specifically examine the DNA methylation status within the heterochromatic region [1]. The group further concludes that transposon DNA methylation can be guided by histone methylation. Results from Johnson and colleagues also show that loss of DNA methylation in a *ddm1* mutant background results in loss of H3mK9 in the heterochromatic portions of the genome, such as centromeres and silenced transposable elements [2]. Further, loss of DNA and histone methylation derepresses retroelements. Johnson *et al.* also tested H3 histone methylation in a *chromomethyltransferase 3* (*cmt3*) background. *CMT3* encodes a methyltransferase that favors CpNpG targets in *Arabidopsis* [4,5]. In a *cmt3* mutant background, H3mK9 levels and gene expression do not positively correlate in two heterochromatic retrotransposon targets.

Histone and DNA methylation cross talk

The results from both groups are consistent with emerging evidence of cross talk between histone and DNA methylation. In *Arabidopsis*, loss of H3 K9 methylation results in reductions in a specific subset of DNA methylation targets [6]. In *Neurospora*, loss of histone methylation results in complete loss of genomic DNA methylation [7]. The results suggest that H3mK9 guides DNA methylation to its appropriate placement within the genome. *DDM1* encodes a gene similar to SWI–SNF factors, the engines of large macromolecular protein complexes that mediate DNA–protein dynamics, in some cases with transcriptional consequences. Mismanagement of H3mK9 in a *ddm1* mutant background can lead to loss of cytosine methylation, although loss of DNA methylation can affect the subsequent chromatin packaging. Grendel and colleagues argue that in certain targets, such as retrotransposons, histone methylation markers form the initial signal to recruit DNA methylation, although it is still possible that DNA methylation guides histone methylation or that they are guided in concert by an underlying factor such as DDM1. Johnson *et al*. conversely argue that H3mK9 loss comes from transcriptional activation that causes H3mK4 replacement. Their results demonstrate that H3mK9 is not sufficient for gene silencing of the examine targets. The underlying mechanism of how histone and DNA methylation patterns are established has still to be agreed.

Small RNAs enter the mix

Another emerging mechanism of how methylation patterns are established comes from small RNA species (called small interfering RNA, or siRNA) associated with post-transcriptional gene silencing (PTGS) also called RNA interference (RNAi), to guide DNA and histone modifications to the appropriate heterochromatic region. According to Judy Bender (Johns Hopkins University, Baltimore, MD, USA) 'There's been a lot of literature in the past several years showing a connection between aberrant RNAs and DNA methylation in plants (RNA-directed DNA methylation), but it's been a big unknown as to whether this is a plant-specific mechanism.' Thomas Volpe and colleagues now show that these small RNAs target heterochromatic transcriptionally silenced genomic regions in *Schizosaccharomyces pombe*, and the authors argue that the processed RNAs signal H3K9 methylation [8]. *S. pombe* mutants in RNAi machinery relieved silencing of a marker gene in the centromeric region, and there was an increase in K4 and a decrease in K9 H3 histone methylation. *S. pombe* lacks DNA methylation, so it is difficult to discern if this mechanism is relevant to DNA-methylated plants. However, Marjori Matzke at the Austrian Academy of Sciences (Salzburg, Austria) called the papers 'a significant step forward in understanding how epigenetic changes are targeted to specific regions of the genome.' Volpe *et al*. argue that the processed RNAs make a signal for histone H3 K9 methylation in heterochromatic regions such as the centromere. In organisms that have DNA methylation systems (plants, mammals), the K9-modified chromatin region could then further signal DNA methylation. This model ties in with the 'histone modification drives DNA methylation' model in the paper by Grendel *et al*. [1]. Conservation between plants and a fungal system suggests that there might be a general cross-kingdom conservation extending to animals, which has recently been demonstrated in *Drosophila* PTGS [9].

Gendrel *et al*. [1], Johnson *et al.* [2] and Volpe *et al*. [8] show how DNA and histone methylation, chromatin and siRNAs potentially link together to target heterochromatic regions. Available antisense and mutations in the major DNA methyltransferase in *Arabidopsis* should be useful to determine if DNA methylation itself or *ddm1* loss of function alters patterns of histone methylation [10]. Also, *Arabidopsis* has a battery of characterized mutants defective in various components of the RNAi machinery, which will allow researchers to test whether siRNA affects DNA or histone methylation patterns [11]. The three partners might form a new DNA–RNA–protein interface used in targeting epigenetic silencing [12].

References

1 Gendrel, A.V. *et al.* (2002) Dependence of heterochromatic histone H3 methylation patterns on the *Arabidopsis* gene DDM1. *Science* 297, 1871–1873

2 Johnson, L. *et al.* (2002) Interplay between two epigenetic marks. DNA methylation and histone H3 lysine 9 methylation. *Curr. Biol.* 12, 1360
3 Richards, E.J. and Elgin, S.C. (2002) Epigenetic codes for heterochromatin formation and silencing: rounding up the usual suspects. *Cell* 108, 489–500
4 Bartee, L. *et al.* (2001) *Arabidopsis cmt3* chromomethylase mutations block non-CG methylation and silencing of an endogenous gene. *Genes Dev.* 15, 1753–1758
5 Lindroth, A.M. *et al.* (2001) Requirement of CHROMOMETHYLASE3 for maintenance of CpXpG methylation. *Science* 292, 2077–2080
6 Jackson, J.P. *et al.* (2002) Control of CpNpG DNA methylation by the KRYPTONITE histone H3 methyltransferase. *Nature* 416, 556–560
7 Tamaru, H. and Selker, E.U. (2001) A histone H3 methyltransferase controls DNA methylation in *Neurospora crassa*. *Nature* 414, 277–283
8 Volpe, T.A. *et al.* (2002) Regulation of heterochromatic silencing and histone H3 lysine-9 methylation by RNAi. *Science* 297, 1833–1837
9 Pal-Bhadra, M. *et al.* (2002) RNAi related mechanisms affect both transcriptional and posttranscriptional transgene silencing in *Drosophila*. *Mol. Cell* 9, 315–327
10 Richards, E.J. (1997) DNA methylation and plant development. *Trends Genet.* 13, 319–323
11 Vaucheret, H. *et al.* (2001) Post-transcriptional gene silencing *in plants*. *J. Cell Sci.* 114, 3083–3091
12 Dernburg, A.F. and Karpen, G.H. (2002) A chromosome RNAissance. *Cell* 111, 159–162

1360-1385/03/$ - see front matter
doi:10.1016/S1360-1385(03)00005-0

Letters

Ligand mimicry? Plant-parasitic nematode polypeptide with similarity to CLAVATA3

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The importance of peptides in plant intercellular signaling has become apparent during the past decade. Among recently identified peptide signals is CLAVATA3 (CLV3), which is involved in cell-fate determination in the shoot apical meristem of *Arabidopsis*. There is evidence that CLV3 is a ligand for CLAVATA1 (CLV1), a receptor kinase with an extracellular domain containing leucine-rich repeats (LRRs) [1]. The *Arabidopsis* genome contains a large gene family, called *CLE* for *CLAVATA3/ESR-related*, encoding polypeptides with similarity to CLV3. These small polypeptides are characterized by a short, C-terminal motif and an N-terminal signal peptide or signal anchor [2]. Similar sequences are encoded by expressed sequence tag (EST) clones from various plants.

Using motif-based database search methods, we have discovered sequence similarity between the CLE polypeptides and an esophageal gland cell polypeptide from *Heterodera glycines*, the soybean cyst nematode (Fig. 1). The sequence similarity between the plant polypeptide family and the *H. glycines* polypeptide, here referred to as HgCLE, was evident from a position-specific iterative BLAST (PSI-BLAST) [3] database search with the CLV3 sequence. Furthermore, the motif-discovery tool MEME [4] was applied to the CLE sequences, and the resulting file was used as input to the MAST algorithm [5] in a database search. The high-scoring sequences were CLV3-like plant polypeptides, but also included HgCLE.

H. glycines is a sedentary plant-parasitic nematode. The infective juvenile stage penetrates the root and migrates to a site near the vascular tissue to establish a permanent feeding site. The nematode induces the transformation of plant cells into metabolically active feeding cells. Secretions from the esophageal gland cells of the nematode are released through the stylet, a mouth spear that is used to pierce plant cell walls. These secretions are thought to contain the substances that cause the transformation of root cells by altering gene expression in the cells [6].

HgCLE is a hypothetical polypeptide predicted from cDNA sequences cloned from esophageal gland cells [7,8]. The cDNA clone hybridized to genomic DNA of *H. glycines*, and expression of the gene was specifically detected in the dorsal esophageal gland cell of parasitic stages of *H. glycines* [7]. The dorsal gland cell is the predominate gland of the parasitic stages of *H. glycines* [6]. HgCLE contains the C-terminal motif and the N-terminal signal peptide that characterize the CLE sequences (Fig. 1). Predicted signal peptide cleavage produces a polypeptide of 117 amino acids, but further processing cannot be excluded. It has yet to be determined whether the CLE polypeptides are processed subsequent to signal peptide cleavage [2]. Indeed, several EST clones from wheat encode sequences containing multiple CLE motifs (Fig. 1). The striking resemblance to a polyprotein precursor could suggest that the conserved motif constitutes the mature CLE peptides.

Based on the similarity between a bioactive plant peptide and a plant-parasite polypeptide, we hypothesize that the nematode has co-opted the plant signaling peptide for parasitic modification of host

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