Transcriptional gene silencing in plants: targets, inducers and regulators

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Gene silencing can occur either through repression of transcription, termed transcriptional gene silencing (TGS), or through mRNA degradation, termed post-transcriptional gene silencing (PTGS). Initially, TGS was associated with the regulation of transposons through DNA methylation in the nucleus, whereas PTGS was shown to regulate virus infection through double-stranded RNA in the cytoplasm. However, several breakthroughs in the field have been reported recently that blur this neat distinction. First, in plants TGS and DNA methylation can be induced by either dsRNA or viral infection. Second, a mutation in the plant *MOM* gene reverses TGS without affecting DNA methylation. Third, in *Caenorhabditis elegans* mutation of several genes that control RNA interference, a form of PTGS, also affect the regulation of transposons. TGS and PTGS, therefore, appear to form two alternative pathways to control incoming, redundant and/or mobile nucleic acids.

Genetic transformation of many eukaryotes has revealed an unexpected finding; the loss of mRNA encoded either by the introduced transgene or by the transgene and endogenous homologous gene(s). These gene-silencing phenomena are termed METHYLATION INDUCED PREMEIOTICALLY (MIP; see GLOSSARY), REPEAT-INDUCED POINT MUTATION (RIP) or QUELLING in fungi[1], RNA INTERFERENCE (RNAi) or COSUPPRESSION in animals[2], and TGS, PTGS, REPEAT-INDUCED GENE SILENCING (RIGS), cosuppression or VIRUS-INDUCED GENE SILENCING (VIGS) in plants[3,4]. MIP, RIP, TGS, RIGS and some cases of cosuppression occur at the transcriptional level (i.e. transcription is prevented), whereas quelling, RNAi, PTGS, VIGS and some cases of cosuppression operate at the post-transcriptional level (i.e. mRNA is degraded after transcription) (Table 1). Recently, some of the genes that control these transgene-induced silencing phenomena have been identified and found to be related to genes implicated in human genetic diseases[5–7] (Table 2). Plants such as *Arabidopsis thaliana* (the genome of which is now complete) are excellent tools to study gene silencing. Numerous targets that undergo silencing are known in this plant (including endogenous genes, transposons, viruses and transgenes) and screens for mutants that release silencing can be applied to any of these. This review focuses on TGS, particularly on the recent reports showing that TGS, like PTGS, can be triggered by either dsRNA or viruses and released without changing the methylation pattern of the silenced gene.

Silencing of transgenes in plants

cis-TGS

In plants, transgenes insert into the genome apparently at random by illegitimate recombination so that the number of inserted copies, their chromosomal location and their local arrangement (tandem insertion, rearrangements, etc.) vary between one transformant and another. An inverse correlation between copy number and the level of gene expression has been reported, which suggests that increasing the number of copies of a particular gene can lead to gene silencing. Indeed, most of the well-characterized loci that undergo TGS contain multiple copies of a transgene[8–10]. As

Glossary

***cis*-TGS**
TGS event affecting single or multiple copies inserted at one locus (i.e. it does not require the presence of homologous sequences in the genome).

Cosuppression
This generic term is used in plants, *Caenorhabditis elegans* and *Drosophila* to describe the reciprocal silencing of transgenes and (partially) homologous endogenous genes. Cosuppression occurs either at the transcriptional level, when the homology is within the promoter, or at the post-transcriptional level, when the homology is within the coding sequence.

Methylation induced premeiotically (MIP)
A form of reciprocal *trans*-TGS observed in the fungus *Ascobolus immersus* resulting in a block of transcription elongation within the duplicated sequences.

Paramutation
A form of *trans*-TGS event induced by allelic silent copies. In some cases the allele that undergoes *trans*-TGS itself becomes able to trigger *trans*-TGS of another active copy.

Post-transcriptional gene silencing (PTGS)
A form of gene silencing identified by a combination of transcription run-on and northern blotting analyses showing that RNA encoded by sense transgenes are degraded after transcription.

Quelling
A form of PTGS induced by sense transgenes in the fungus *Neurospora crassa.*

Repeat-induced gene silencing (RIGS)
A *cis*-TGS event induced by the insertion of multiple copies of a transgene at one locus. RIGS is abolished by reducing copy number.

Repeat-induced point mutation (RIP)
A phenomenon similar to MIP that is associated with point mutation in the duplicated sequences in the fungus *N. crassa.*

RNA interference (RNAi)
A form of PTGS induced by injection of double-stranded RNA in animals or by expression of dsRNA by transgenes with a panhandle structure in animals and plants.

Transcriptional gene silencing (TGS)
A form of silencing identified by run-on experiments showing that transcription initiation is blocked in the nucleus.

***trans*-TGS**
Unidirectional TGS event affecting an active locus (i.e. a locus that does not undergo *cis*-TGS spontaneously) induced by allelic, ectopic or extra-chromosomic homologous sequences.

Virus-induced gene silencing (VIGS)
A form of PTGS that is induced by viruses rather than transgenes.

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Table 1. Main characteristics of the known TGS and PTGS phenomena in plants

Main characteristics	TGS[a]			PTGS		
	***cis*-TGS**	***trans*-TGS**	**VIGS**	**sense-PTGS**	**RNAi**	**VIGS**
Target(s)	transgenes	(trans)genes	transgenes	(trans)genes	(trans)genes	(trans)genes
Presence of target nuclear RNA?	no	nd	nd	yes	nd	nd
Presence of target cytoplasmic RNA?	no	no	reduced	reduced	reduced	reduced
Methylation of target(s)	yes (transgene)	yes (transgene) nd (endogene)	yes (transgene)	yes (transgene) no (endogene)	nd (transgene) nd (endogene)	yes (transgene) no (endogene)
Inducing agent(s)	surrounding chromatin or transgene repeats	transgenes expressing promoter dsRNA	viruses carrying promoter sequences	transgene expressing sense (aberrant) RNA	transgene expressing ORF dsRNA	viruses carrying ORF sequences
Transcription of inducer required?	–	yes	yes	yes	yes	yes
Production of a systemic signal?	nd	nd	no	yes	nd	yes
Release by non-homologous viruses?	no	nd	nd	yes	nd	yes
Mutations that release silencing	*ddm1*, *som2*, *som3*, *som6*, *mom1*, *hog1*, *sil1*, *met1*	nd	nd	*ago1*, *sgs1*, *sgs2*/*sde1*, *sgs3*, *sde2*, *sde*3, *sde4*, *ddm1*, *met1*	nd	nd
Mutations that do not release silencing	*sgs1*, *sgs2*, *sgs3*	nd	nd	nd	nd	*sgs2*/*sde1*

[a]See glossary for silencing terms.

Abbreviations: nd, not determined; ORF, open reading frame.

transgenes within these loci are hypermethylated and have an increased resistance to DNase I digestion[11], it is assumed that TGS occurs in *cis* as a result of pairing between closely linked copies that leads to the formation of secondary DNA structures that attract methylation and heterochromatin components. However, these analyses did not determine whether hypermethylation is a cause or a consequence of TGS. Occasionally, single copies of a transgene are subject to TGS (Ref. 12). It is assumed that this results from large discrepancies between the GC content of the transgene and that of the surrounding genomic sequences[12]. Indeed, introduction of orthologous genes from maize (a monocotyledon) or gerbera (a dicotyledon) into petunia (a dicotyledon) leads to silencing, an effect that is stronger with the maize gene[13].

trans-TGS

In the examples described above, TGS occurs in *cis*, so that copies of the transgene inserted at one locus are affected. However, transgenes that are active initially can be silenced in *trans* by another transgene introduced by either transformation or crossing. Indeed, there are reports of TGS and *de novo* methylation of one transgene that is mediated by a second transgene driven by an identical promoter (reviewed in Ref. 3). The mechanism by which *trans*-TGS occurs in these cases is not known. It was suggested that interaction of homologous sequences (DNA–DNA pairing) leads to the transfer of a silent chromatin state from one locus to the other. Alternatively, the involvement of a silencing RNA produced by one locus has also been invoked. Recently, dsRNA that contains promoter sequences has been demonstrated to trigger TGS and *de novo* DNA methylation of the corresponding target transgene (or endogenous gene), indicating that *trans*-TGS can operate through an RNA intermediate[14]. Double-stranded RNA containing the target promoter sequences were produced using a transgene with a panhandle structure driven by another promoter. This dsRNA is partially cleaved into small RNAs ~23 nucleotides in length. Such small RNAs are also observed when dsRNA corresponding to open reading frames (ORFs) are introduced in *C. elegans* (leading to silencing of the homologous endogenous genes by RNAi)[15] or when dsRNA corresponding to ORFs are expressed by transgenes in plants (leading to silencing of both the transgenes and of the homologous endogenous genes by PTGS)[16]. Both TGS and PTGS can, thus, be initiated by the same dsRNA-degradation pathway. However, TGS occurs when dsRNA contain copies of promoter sequences whereas PTGS occurs when dsRNA include ORFs. This indicates that although the degradation of dsRNA is common to both pathways, other steps exist that are specific to each.

trans-TGS mediated by viruses

Links between viral infection and gene silencing have been discovered recently (reviewed in Refs 3 and 4). Plants can escape from viral infection by specifically

Table 2. Similarity between functions of genes implicated in human genetic diseases and those controlling silencing phenomena

Transgene-induced silencing phenomenon[a]	Organism	Gene	Function(s)	Human genetic disease	Gene
Quelling	*Neurospora*	*QDE-3*	DNA helicase	Werner syndrome	*WRN*
RNAi	*C. elegans*	*MUT-7*	3′→5′ RNase	Werner syndrome	*WRN*
TGS	*Arabidopsis*	*DDM1*	SWI2/SNF2 chromatin remodeling	ATR-X syndrome	*ATRX*
MIP	*Ascobolus*	*Masc1*	DNA methyltransferase	ICF syndrome	*DNMT3B*
TGS	*Arabidopsis*	*MET1*	DNA methyltransferase	ICF syndrome	*DNMT3B*

[a]See GLOSSARY for silencing terms.

degrading viral RNA after a preliminary period of infection. This phenomenon, known as recovery, closely resembles PTGS because it is systemic. It can be observed with both DNA viruses such as cauliflower mosaic virus (CaMV), which replicate in the nucleus[17] and RNA viruses such as tobacco rattle virus or tomato black-ring virus, which replicate in the cytoplasm[18]. Although other viruses, including potato virus X (PVX), an RNA virus, do successfully infect plants, small RNAs (23 nucleotides in length) homologous to the virus are found in infected plants[16]. Thus, although the plants respond to infection by initiating the silencing process, they do not succeed in eliminating the virus completely. Infection of plants by CaMV can also trigger TGS of integrated transgenes that are driven by the 35S promoter of CaMV and PTGS of transgenes encoding RNA containing the CaMV 35S RNA sequences[17]. Similarly, infection with a recombinant PVX virus that contains the promoter sequence of an integrated transgene triggers TGS of the transgene whereas infection of plants by a recombinant PVX virus that contains even part of the ORF of a transgene triggers PTGS of this transgene[19]. Thus, as with transgenes encoding dsRNA, both DNA and RNA viruses seem to be able to trigger either TGS or PTGS of a transgene, depending on whether they share homology with its promoter or its coding sequence. However, in contrast to the transgene situation, where the dsRNA is synthesized in the nucleus, the RNA generated by RNA viruses in the cytoplasm (the compartment where RNA viruses replicate) must enter the nucleus to initiate DNA methylation and TGS.

Genes that control TGS in plants

To understand the mechanisms underlying TGS, mutants that were unable to establish or maintain gene silencing were sought using genetic screens for suppressors of TGS in *Arabidopsis* TGS-transgenic lines. One line (A) contained a complex locus containing multiple copies of the 35S promoter driving the gene encoding hygromycin phosphotransferase (hpt) silenced in *cis*[9]. In eight mutants, denoted *som,* the usually silent, hypermethylated locus A was hypomethylated and reactivated (Table 3). Like *ddm1* and *met1* (also known as *ddm2)* mutants (which were isolated based on the demethylation of centromeric and rDNA repeats)[20], centromeric and rDNA repeats were hypomethylated in the eight *som* mutants[9]. Allelism tests classified *som1, som4* and *som5* as alleles of *ddm1* and *som2* as a mutant affected in another gene. *som3*, *som6*, *som7* and *som8* could not be classified due to slow re-silencing of the A locus in outcrosses[9]. Sequencing of the cloned *DDM1* gene[21] in *som* mutants generated by fast neutron irradiation revealed mutations that confirmed that *som4*, *som5*, *som7* and *som8* are alleles of *ddm1* (Ref. 21). *DDM1* encodes a protein that has strong similarities to the SWI2/SNF2 chromatin-remodelling proteins. Reactivation of the A locus in *ddm1/som* mutants is assumed to result from changes in chromatin structure rather than hypomethylation because the A locus remains silenced even though it becomes hypomethylated in *met1* plants[9]. This hypothesis was reinforced by the identification of the *mom1* mutant in which the A locus is reactivated although the methylation state is not altered[22]. In addition, the *mom1* mutant does not affect methylation of centromeric repeats. The *MOM* gene encodes a novel nuclear protein with a region related to SWI2/SNF2 proteins. Like DDM1, MOM probably participates in TGS by remodelling chromatin. However, unkile DDM1, MOM is not absolutely required to make DNA accessible to the methylation machinery, which suggests it could act downstream of DDM1. These results also suggest that MOM could act downstream of MET1, and that MET1 could be dispensable to allow MOM to trigger TGS at some loci (Fig. 1).

An independent screen for TGS suppressors was performed using a line that has three linked transgenes (CHS, npt and hpt) at one locus (locus C) (Ref. 10). Although crossing the C line with *ddm1* mutants abolished TGS of these three transgenes (Table 3), the genetic screen identified two other loci, named *hog1* and *sil1*. In the *hog1* mutant the three transgenes are hypomethylated and reactivated and, as in *ddm1* and *som* mutants, rDNA repeats are hypomethylated, although to a lesser extent. However, *hog1* is not a *ddm1* allele[10]. The *sil1* mutant reactivates only two of the transgenes at the C locus (npt and hpt). Like *mom1*, *sil1* does not affect methylation of the silenced transgene locus and of the rDNA repeats. However, *sil1* is not a *mom1* allele[22]. *SIL1,* therefore, encodes a new component of the TGS pathway that acts downstream or is independent of *MET1*.

Finally, the effect of three PTGS suppressors was tested on loci A and C that usually undergo TGS (Table 3). None of the *sgs1*, *sgs2* and *sgs3* mutants abolished *cis*-TGS (Refs 23,24). Because the resistance

Table 3. Expression and methylation of endogenous and transgene loci in wild type, TGS and PTGS *Arabidopsis* mutants

Locus[a]	wt	Mutants[b]										
		ddm1	*som2*	*som3*	*som6*	*mom1*	*hog1*	*sil1*	*met1*	*sgs1*	*sgs2*	*sgs3*
centromer	Me	me	me	me	me	Me			me	Me	Me	Me
rDNA	Me	me					me	Me	me	Me	Me	Me
A (hpt)	TGS Me	NS me	NS me	NS me	NS me	NS Me			TGS me	TGS	TGS	TGS
C (npt)	TGS Me	NS me					NS me	NS Me		TGS	TGS	TGS
6b5 (GUS)	TGS Me	NS me	NS	NS	NS	NS	NS		NS me	TGS	TGS	TGS
PAI2	TGS Me	NS me							NS me			
MEA	TGS	NS										
Tar17	TGS Me	NS me										
Mu-like	TGS Me	NS me										
TSI	TGS Me	NS me	NS me	NS me	NS me	NS Me	NS me	NS	NS me	TGS	TGS	TGS
SUP	NS me	TGS Me							TGS Me			
AG	NS me	TGS Me							TGS Me			

[a]centromer (180-bp unit) and rDNA are highly repetitive sequences. Only methylation was checked for these. A, C and 6b5 are transgene loci. *PAI2, MEA, SUP* and *AG* are endogenous genes. *Tar17, Mu-like* and TSI are transposon(-like) sequences.

[b]*ddm1, som2, som3, som6, mom1, hog1, sil1* and *met1* are TGS mutants. *sgs1, sgs2* and *sgs3* are PTGS mutants.

Abbreviations: Me, hypermethylated; me, hypomethylated; NS, non-silenced; PTGS, post-transcriptional gene silencing; TGS, transcriptional gene silencing.

to RNA viruses that replicate in the cytoplasm is also altered in these PTGS mutants[24], the absence of reactivation of transgene loci that undergo TGS in the nucleus is not really a surprise. Other suppressors of PTGS, named *sde* have been identified recently[25]. However, their effect on *cis*-TGS has not been determined. Whether *sgs* and *sde* PTGS mutants relieve the *trans*-TGS mediated by promoter dsRNA or viral infection needs to be tested to determine how many steps *trans*-TGS shares with PTGS. Whether TGS mutants could abolish the *trans*-TGS mediated by promoter dsRNA or by viruses also needs to be tested. Indeed, initation of *trans*-TGS could require the PTGS machinery, whereas its maintenance could require the TGS machinery described above. A genetic screen for suppressors of TGS induced by dsRNA should allow additional components of this type of TGS to be identified[14].

Endogenous targets of TGS in plants

Transgenes and transgenic plants are powerful tools by which to identify TGS mutants, but they provide a limited view of the natural role of transcriptional repression. Nevertheless, TGS mutants derived from transgenic lines can be used to identify endogenous sequences regulated by TGS (Table 3). It was expected that endogenous sequences would be regulated by TGS because some of the TGS mutants (*ddm1* and *met1*) have developmental abnormalities[20] (Fig. 2). Sequences that are deregulated in TGS mutants can be classified into two groups, sequences that are naturally silenced by TGS in wild-type plants and reactivated in TGS mutants, and sequences that are naturally expressed in wild-type plants and (unexpectedly) silenced in TGS mutants.

Silenced sequences reactivated in TGS mutants

Three types of sequences have been identified that are reactivated in TGS mutants: endogenous genes, transposons and sequences of unknown functions. Within the small family of genes that encode phosphoribosyl anthranilate isomerase (PAI), one functional member gene (*PAI2*) undergoes TGS and is hypermethylated in certain ecotypes. This gene is reactivated and hypomethylated in *ddm1* (Ref. 26). The parentally imprinted gene *MEDEA* (*MEA*) is inherited in a silenced state on the paternally transmitted chromosome of wild-type plants. This gene is also reactivated in *ddm1* (Ref. 27). Silent transposons, including the retroelement *Tar17* and mutator-like elements, are also hypomethylated and transcriptionally active in *ddm1* plants (Ref. 28; F. Singer and R. Martienssen, unpublished). Although it is not known whether these transposons are also reactivated in the other TGS mutants, it is assumed that the reactivation of transposons could account for at least part of the developmental abnormalities induced in imbred generations of TGS mutants[20].

Transcriptionally silent information (TSI) sequences were identified by subtractive hybridization of cDNA from wild-type plants and the *mom1* mutant (which has no developmental abnormality)[29]. Transcription of TSI initiates in the middle of degenerate members of the Athila retroelements family. It is assumed that TSI does not encode a protein and the function of TSI RNA is not known. Interestingly, RNA from TSI occurs in all known TGS mutants (*ddm1*, *mom1*, *hog1*, *sil1* and all *som* mutants), as well as in the methylation mutant *met1/ddm2*. In addition, different expression patterns of TSI are observed in the different mutants (up to four differently sized transcripts can be detected in various ratios)[29]. The variable spectrum of reactivation of TSI elements in different mutants is consistent with the variable spectrum of reactivation of other loci that undergo TGS. Indeed, transgene locus A is not reactivated in *met1/ddm2* mutants whereas TSI, *PAI2* and the transgene locus 6b5 that contains the 35S–*glucuronidase* (*GUS*) reporter gene[22,30] are

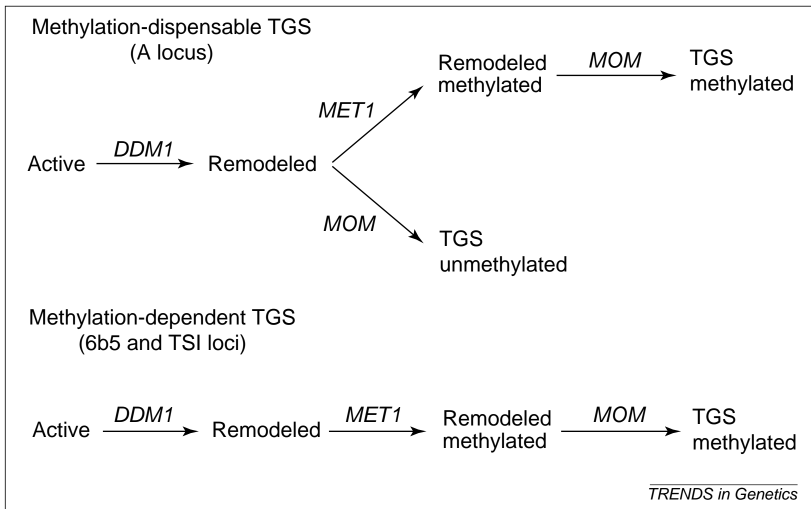

Fig. 1. Methylation-dispensable versus methylation-dependent transcriptional gene silencing. Expression and methylation of A, 6b5 and transcriptionally silent information (TSI) loci in *ddm1*, *met1* and *mom1* backgrounds suggest the existence of two alternative modes of transcriptional gene silencing (TGS).TGS and hypermethylation of the three loci are abolished in *ddm1*. However, in *met1*, although all three loci are hypomethylated, locus A still undergoesTGS. Furthermore,TGS but not hypermethylation of the three loci is abolished in *mom1*. These results suggest that *DDM1* is required for DNA to be accessible to the methylation machinery and that *MOM* acts downstream of both *DDM1* and *MET1*. They also suggest that *MET1* is required forTGS of the 6b5 andTSI loci, whereas it is dispensable for TGS of locus A (A is silenced in both wild-type plants, where it is hypermethylated, and in *met1* plants, where it is hypomethylated).

(Table 3; Refs 9,29,30; J. Bender, unpublished results). These differences suggest that the number of components participating in the transcriptional control of a transgene locus is variable. Either the exact location of the chromosomal or subnuclear compartment of the transgene locus or its genomic organization (multicopy, direct or inverted repeat) could determine which TGS components are required to repress transcription. Genetic screens based on the reactivation of *PAI2*, *MEA*, TSI or 6b5 loci should allow the identification of additional (specific) components of TGS in *Arabidopsis*.

Genes silenced in TGS mutants

Two endogenous genes define the second group of sequences deregulated in TGS mutants. The floral developmental genes *SUPERMAN (SUP)* and *AGAMOUS (AG)* are occasionally hypermethylated and silenced in *ddm1* and *met1* mutants, although the majority of the genome becomes hypomethylated in these mutants[31]. The formation of silenced *sup* and *ag* alleles suggests a dramatic redistribution of methylation in globally hypomethylated cells. This could reflect chromatin changes within the *SUP* and *AG* loci that make them more accessible to the methylation machinery. Alternatively, it could reflect the derepression of particular methyltransferase genes that are expressed at very low level in wild-type plants. Interestingly, this phenomenon resembles the hypermethylation of human tumor-suppressor genes observed during the early stages of cancer when there is a general genome-wide decrease in methylation[32]. Therefore, understanding the mechanism by which particular genes become hypermethylated in plants could help in cancer research.

TGS in evolution and development

Endogenous genes that undergo heritable TGS have been described in other plant species, including toadflax and maize, in hybrids and in allopolyploids. Although the effect of TGS mutations cannot be tested in these organisms (all TGS mutants have been isolated in *Arabidopsis*), their study is a rich source of information on the role of TGS during evolution. One of the oldest known mutants in plants (a mutant toadflax impaired in floral symmetry) has a hypermethylated, silenced epiallele of the *Lcyc* gene[33]. The phenotype was described 250 years ago, reflecting how stable the inheritance of TGS is. Furthermore, endogenous genes that undergo TGS spontaneously can sometimes trigger efficient and heritable *trans*-TGS of allelic partners on the homologous chromosome. This phenomenon, called PARAMUTATION, was described in maize during the 1950s (Ref. 34). More recently, it has been shown that transgenes can also undergo paramutation[12]. During crossing, paramutable (active) alleles become silenced in the presence of paramutator (silenced) alleles; neutral (active) alleles, however, remain unchanged in the presence of paramutator alleles. Because some paramutable alleles can be converted into paramutator alleles during paramutation (they become able to silence paramutable alleles in the next cross), one would expect the paramutator alleles to invade the entire population of plants and the paramutable alleles to disappear. This is not observed, however. Genetic screens for suppressors of paramutation in maize have led to the isolation of several mutants[34]. The cloning of the corresponding genes will determine whether this phenomenon shares common step(s) with TGS in *Arabidopsis*. Finally, a natural form of silencing, called nucleolar dominance, probably reflects evolutionary strategies. Nucleolar dominance is a phenomenon observed in hybrids or allopolyploids in which nucleoli form on chromosomes inherited from only one of the parents. It is supposed to result from selective silencing of one set of rRNA genes through chemical modification of chromatin. However, the mechanism responsible for initially discriminating among the parental sets of rRNA genes remains unclear[35].

Conclusions and perspectives

Until recently, TGS and PTGS were considered as separate pathways. TGS was thought to regulate mainly transposons and (accidentally) transgenes that mimic transposons because of their position, structure or redundancy. By contrast, PTGS was said to regulate viral infection and (accidentally) transgenes that encode some types of aberrant RNA that mimic viral RNA. Transgene methylation, which is associated with both TGS and PTGS, was not considered to link these two phenomena because, in *sil1* and *mom1* mutants, TGS can be abolished without affecting methylation[10,22] and PTGS can be abolished without affecting methylation in virally infected plants[19]. The major breakthrough in the distinction between TGS and PTGS came with the discovery that viruses and transgenes encoding dsRNA induce either TGS or PTGS of a homologous transgene

Fig. 2. Developmental abnormalities induced in *met1* plants. A 6-week-old wild-type *Arabidopsis* plant of the Columbia ecotype (left) and three plants of the same age transformed independently with the *asMET1* transgene (right). Expression of this transgene, which encodes an antisense RNA directed against *MET1*, results in developmental abnormalities, such as delay in flowering, stem fasciation and sterility. The *asMET1* transgene was kindly provided by J. Finnegan (CSIRO, Canberra, Australia).

depending on whether they share sequence homology with its promoter or ORF sequence[14,17,19]. The discovery that mutations in several genes controlling RNAi in *C. elegans* also affect the regulation of transposons[36,37] suggested that TGS and PTGS could form alternative, nonexclusive ways to regulate the same elements (for instance, transposons).

The data reported in this review indicate that *cis*-TGS requires a variable set of chromatin and/or methylation proteins (Fig. 1, Tables 1 and 3). Furthermore, as *trans*-TGS, like PTGS, involves the participation of RNA (dsRNA or virus RNA), it probably requires these plus other proteins. One would, therefore, predict that both common and specific components of TGS and PTGS exist. For instance, *SGS2* and *SGS3* (which encode a protein similar to RNA-dependent RNA polymerase and a protein of unknown function with no known homologues, respectively) control PTGS but not *cis*-TGS (Ref. 24). Whether these proteins also control *trans*-TGS needs to be determined. The *AGO1* gene, which encodes a protein similar to the translation initiation factor eIF2C, also controls PTGS (Ref. 38) although whether this protein has a role in *cis*- or *trans*-TGS is not known. The proteins encoded by the *MET1* and *DDM1* genes (which encode the major DNA methyltransferase and a protein similar to the SWI2/SNF2 chromatin-remodelling proteins , respectively) control *cis*-TGS (Refs 9, 10, 29, 30; J. Bender *et al.* , unpublished). We showed recently that PTGS is abolished stochastically in *ddm1* and *met1* mutants, indicating that *DDM1* and *MET1* participate both in *cis*-TGS and PTGS[30]. Whether these genes also control *trans*-TGS needs to be determined. Finally, *MOM* (which encodes a newly identified nuclear protein with a SWI2/SNF2 motif) controls *cis*-TGS (Ref. 22). Whether it controls *trans*-TGS or PTGS is not known.

As the number of genetic screens for TGS and PTGS mutants increase, knowledge of the common and specific components of these processes should also increase. Analysis of the consequences of mutation of candidate genes should also be helpful. For example, expression of the TGS and PTGS targets described above should be determined in the *pickle (pkl)* and *curly leaf (clf)* mutants of *Arabidopsis*. These mutations impair genes that encode orthologs of eukaryotic *CHD3* genes implicated in chromatin-mediated repression[39] and the polycomb group of genes that are involved in chromatin-mediated repression of homeotic genes in *Drosophila*[40], respectively. Thousands of tagged *Arabidopsis* mutants have been generated by insertion of T-DNA or transposons, and the sequences that surround the junctions of these inserted elements with plant DNA are becoming available. Thus, within the next few years it will be possible to identify mutants and to determine the role of each plant gene. In particular, this method will allow determination of the role of proteins encoded by multigene families. For example, several genes encoding DNA methyltranferases exist in *Arabidopsis*[41]. Multiple mutants, made by successive crosses between individual tagged mutants, will allow the expression of TGS and PTGS targets to be compared in these different genetic backgrounds. Such an approach is currently being used to examine the role of chromatin remodelling proteins by the authors of the Plant Chromatin Database (http://ag.arizona.edu/chromatin/).

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Nuts and bolts of psychiatric genetics: building on the Human Genome Project

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Schizophrenia and bipolar affective disorder are chronic, disabling illnesses that together affect 2% of the population. Genetic factors are known to be important in their development, but there are, as yet, no confirmed susceptibility genes. Here we discuss important issues in terms of alternative genetic strategies (linkage, association and/or cytogenetics) in the identification of candidate genes for the major psychoses. We discuss the impact of the Human Genome Project, the role of comparative genetics in finding and testing positional candidates, and the prospects for rational drug design and personalized medicine.

Mental illnesses are among the most common causes of chronic morbidity worldwide[1]. Two severe forms, schizophrenia and bipolar affective disorder (BPAD) each affect around 1 in 100 individuals, often with onset in early adult life. In spite of their high prevalence and decades of research in neurochemistry, neuropathology, neuropsychology, brain imaging and, indeed, genetics, the causes of these conditions remain unknown and treatments mainly empirical.

Both conditions tend to run in families, and the risk to a first-degree relative of an affected person is about ten times that to a member of the general population. Twin and adoption studies indicate that inherited factors are responsible for a major part of this increased risk. For a number of reasons, however, progress towards identifying genes has been difficult. Diagnosing psychiatric illness is imprecise because psychiatric phenotypes are mainly based on symptom profiles reported by patients. The use of standardized diagnostic criteria[2,3] has ensured good reproducibility of diagnoses between researchers, but there is wide overlap of symptoms between schizophrenia, BPAD and unipolar depression (Box 1). In the absence of reliable biological or genetic markers specific for schizophrenia or BPAD, the validity of existing classification remains uncertain.

Approaches to finding genes underlying psychiatric disorders

Linkage and association approaches

In linkage studies, the problem of diffuse diagnostic boundaries is usually met by carrying out analyses

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