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Plants, their organelles, viruses and transgenes reveal the mechanisms and relevance of post-transcriptional processes Leysin, VD, Switzerland, February 25–28, 1999

Julia Bailey-Serres[1], Jean-David Rochaix[2], Michael Wassenegger[3] and Witold Filipowicz[4]

Department of Botany and Plant Sciences, University of California, Riverside, CA 92521, USA, [2]Department of Molecular Biology, University of Geneva, 30 Quai E.Ansermet, CH-1211 Genève, [4]Friedrich Miescher Institute, PO Box 2543, CH-4002 Basel, Switzerland and [3]Fraunhofer Institute, Department Molekulare Biotechnologie, Am Klopferspitz 18A, 82152 Martinsried, Germany

[1]Corresponding author
e-mail: serres@mail.ucr.edu

Introduction

Plants have evolved a plethora of mechanisms to respond to the environment and modulate growth and reproduction. Abiotic signals provided by light, temperature and the availability of water, oxygen and nutrients are controlling factors. Many species possess strategies to endure abiotic assaults, and to respond to challenges from bacterial, fungal and viral pathogens. Although these responses are frequently regulated at the transcriptional level, there is extraordinary diversity in the post-transcriptional mechanisms that promote developmental plasticity and adaptation. The phenomenon of silencing of heterologous genes also involves transcriptional and post-transcriptional mechanisms. Current research links gene silencing to ordinary post-transcriptional mechanisms as well as defense of viral RNAs. An EMBO Workshop in Leysin, Switzerland, explored the mechanisms, implications and connections of post-transcriptional processes in plants. Several non-plant scientists also participated and presented an overview of post-transcriptional processes in mammals and yeast.

Nuclear processes: dynamics of organization, intron splicing and mRNA accumulation

Confocal microscopy and *in situ* hybridization procedures have led to enormous progress in the visualization of the spatial organization and dynamics of transcription and RNA-processing machinery in eukaryotic cells. It is well established that the nucleus is a highly organized structure composed of many different territories, subdomains and organelles (Lamond and Earnshaw, 1998). A state-of-the-art account of the nuclear architecture of plant cells was given by P.Shaw (John Innes Centre, Norwich, UK). Mapping of the RNA polymerase I transcription and rRNA processing sites, using different pre-rRNA, snoRNA and nucleolar protein probes, indicates that the plant cell nucleolus is organized as a series of concentric layers in which transcription and successive rRNA processing reactions occur. As in vertebrate cells, plant cells contain coiled bodies (CBs), nuclear organelles of still poorly defined function. Since CB components include several small RNAs, and also proteins related to either their assembly or function, CBs are thought to be involved in snRNP/snoRNP maturation, recycling or transport. Stably transformed cell lines expressing a fusion of the spliceosomal protein U2B″ with GFP allowed the visualization of the dynamic behavior of CBs. CBs were seen to move within the nucleoplasm, fuse together or move from the nuclear periphery to the nucleolus. This is consistent with their proposed role in nuclear transport. *Arabidopsis* lines that express U2B″–GFP have been made, and open the way for a mutagenesis screen for genes controlling CB structure and activity.

The basic mechanism of mRNA splicing and spliceosome assembly in plants resembles that of mammals and yeast with certain distinctions. It is a two-step reaction that involves the formation of a mRNA lariat and spliceosomal U-snRNAs, U-snRNP proteins and splicing factors. Plant intron 5′ and 3′ splice sites are generally similar to their vertebrate counterparts. However, requirements for intron recognition differ from those in other eukaryotes, resulting in the inability of plants to splice heterologous pre-mRNAs. Plant introns are extraordinarily enriched in U residues throughout the intron length, and this property is essential for splicing. Intronic U- or UA-rich elements play an important role in the definition of intron borders and increase the efficiency of splicing (Simpson and Filipowicz, 1996; Brown and Simpson, 1998). W.Filipowicz and co-workers (Friedrich Miescher Institute, Basel, Switzerland) described two families of RNA-binding proteins in *Nicotiana plumbaginifolia* which are associated with nuclear poly(A)$^+$ RNA *in vivo* and have specificity for oligouridylates. Overexpression in protoplasts of one of the proteins, UBP1, stimulates splicing of suboptimal introns and increases steady-state levels of mRNAs, even when they do not contain introns. Hence, UBP1 may target different steps of mRNA metabolism. Although enrichment in pyrimidines of the 3′-splice-site-proximal region in plant introns is not readily apparent, both subunits of the polypyrimidine-tract-binding factor U2AF, first characterized in metazoa, are expressed in plants. Two identified plant isoforms of the large U2AF subunit have specificity for oligouridylates and functionally replace the human factor in the HeLa cell-splicing extract (Domon *et al*., 1998). How the plant splicing machinery distinguishes between the 3′-proximal and upstream U-rich elements during intron recognition remains an intriguing question.

Initial experiments suggested that plant intron splice

sites are selected by the intron definition mechanism, a notion supported by the relatively short length of plant introns and the requirement for intronic UA-rich elements. J.Brown (Scottish Crop Research Institute, Invergowrie, UK) presented experiments that clearly point to an operational exon definition scheme in plants. Hence, as in metazoa, both mechanisms appear to function in plants, the use of a particular pathway depending on the length of the intron, the strengths of the various splicing signals and the properties of adjacent introns. Brown showed that inclusion of very short exons, such as a nine nucleotide exon conserved in many plant invertase genes, depends upon two properly spaced signals located in the upstream intron. These signals, the branch point and the oligo (U) stretch positioned downstream of it, can specify the inclusion of mini-exons as short as one nucleotide. Identification of such exons in gene sequences generated by different genome projects may be a real challenge.

Alternative splicing is a common mechanism of regulating gene expression in metazoan animals. The differential processing is often subject to developmental or tissue-specific control (Lopez, 1998). To date, relatively few examples of alternative mRNA splicing of documented biological significance have been described in plants (Simpson and Filipowicz, 1996; Brown and Simpson, 1998; one of the best documented cases was discussed at the meeting by P.Sivadon, see below). A.Barta (University of Vienna, Austria) reported that alternative splicing may be much more common than originally anticipated. Barta described several proteins in *Arabidopsis* that are similar to the alternative SR-type splicing factors characterized in metazoa. The complexity of this family of factors in *Arabidopsis* approaches that of humans. The two proteins most closely related to the human factor SF2/ASF, atSRp30 and atSRp34, are themselves alternatively spliced and their gene expression and splicing vary in different tissues and during development. Overexpression of atSRp30 in transgenic *Arabidopsis* leads to changes in alternative splicing of several genes and many interesting morphological and developmental abnormalities (Lopato *et al*., 1999).

The rate of mRNA turnover provides a major post-transcriptional step to regulate gene expression. Studies in yeast have led to a description of the general pathways of mRNA degradation, whereas studies in mammalian systems have concentrated on the identification of *cis*- and *trans*-acting factors responsible for controlling the decay of individual mRNAs. In mammals, the short half-lives of many proto-oncogene and cytokine mRNAs are due to the presence of multiple AUUUA-containing motifs in the 3′-untranslated region (UTR; Jacobson and Peltz, 1996). AUUUA repeats also destabilize plant mRNAs when inserted into the 3′-UTR. Another destabilizing motif, DST, which appears to be plant specific, originates from a class of short unstable mRNAs inducible by auxin (Abler and Green, 1996). P.Green (Michigan State University, East Lansing, MI) described selection strategies to isolate mutants of *Arabidopsis* affected in the decay of mRNAs containing DST motifs. Two mutants defective in mRNA decay were isolated from a screen of almost 800 000 EMS-mutagenized M2 plants. Since these recessive mutations increase the abundance of two independent transgene mRNAs and an endogenous DST-containing mRNA, it is possible that they reside in genes encoding *trans*-acting factors. The low frequency of viable mutants that alter the DST-mediated mRNA decay processes may reflect their importance. Green also reported the identification in *Arabidopsis* of several components that catalyze the steps of the general pathway responsible for mRNA degradation in yeast. Three cDNAs encoding Xrn-like 5′3′ exonucleases were characterized and found to resemble more closely the nuclear yeast enzyme Rat1p/Xrn2 than the cytoplasmic enzyme Xrn1p. Enzymes encoded by two of the genes complement yeast *rat1* mutants, suggesting that their products function in the nucleus. Thus far, plants are the only organisms reported to have multiple Rat1p/Xrn2p enzymes and seemingly to lack an Xrn1p.

Translational processes regulated by development, environment and as revealed by viruses

The general mechanisms of mRNA translation are conserved in plants and animals, but notable features of the plant translational apparatus and initiation process were presented. As in other eukaryotes, a $m^7G(5')$ ppp(5′)N-capped (where N is any nucleotide) transcript associates with a complex composed of a cap-binding protein (eIF4E) and its partner (eIF4G). eIF4G assists eIF4A and eIF4B to generate RNA helicase activity, binds to eIF3, which facilitates the recruitment of the 40S ribosomal subunit and interacts with poly(A)-binding protein (PABP) to bring the 5′- and 3′-ends of the transcript into proximity to enhance initiation or re-initiation. In plants, the circularization of the transcript is also promoted by the binding of eIF4B to PABP (Le *et al*., 1997). Interestingly, plants possess an additional cap-binding complex that is composed of the cap-binding protein eIFiso4E and its evolutionarily distinct partner, eIFiso4G. Another cap-binding protein (nCBP) is present in plants and animals, but its function remains unknown (Ruud *et al*., 1998). These cap-binding proteins probably differentially recruit mRNAs for translation.

Formation of the mRNA–cap-binding complex may be modulated by different means in plants than other eukaryotes. In animals, non-phosphorylated eIF4E is apparently sequestered by phosphorylated 4E-binding protein (4EBP). The hyperphosphorylation of 4EBP results in eIF4E phosphorylation and promotes mRNA cap-binding. In yeast, p20, a non-orthologous 4EBP modulates eIF4E activity. Surprisingly, no 4EBP or p20 homologs have been identified in plants. J.Bailey-Serres (University of California, Riverside, CA) reported that eIF4E is additionally phosphorylated in the root tips of maize in response to anoxia, as a result of increased cytosolic Ca^{2+} (Manjunath *et al*., 1999). No modification of eIFiso4E was observed, but eIFiso4G conformation is pH sensitive and the anoxia-stimulated decrease in cytosolic pH is predicted to reduce formation of the mRNA–eIFiso4E–eIFiso4G complex. Hence, initiation may be regulated by cytosolic Ca^{2+} levels and pH. C.Robaglia and co-workers (CEA-Centre de Cadarache, France) searched for an *Arabidopsis* 4EBP using the yeast two-hybrid system and co-immunoprecipitation. They unexpectedly found that the jasmonic acid-induced lipoxygenase-2 that is elevated

in response to wounding competes with eIFiso4G for binding to eIFiso4E. Translation is regulated in response to jasmonic acid (Bailey-Serres, 1999).

One of the most exciting observations reported was that the translation of certain mRNAs may be promoted by specific factors. D.Gallie (University of California, Riverside, CA) rigorously showed that binding of HSP101 to a 25 nucleotide poly(CAA) region of the 5′-UTR of tobacco mosaic virus mRNA stimulates translation. The *Nicotiana tabacum* HSP101 gene complemented the thermotolerance defect of a yeast strain lacking the homologous protein (Hsp104). Use of yeast mutants provided evidence that enhancement of translation by HSP101 involves interactions with eIF3 and only one of the two yeast eIF4Gs (Wells *et al*., 1998). HSP101 levels are regulated during seed development and increase following heat shock or cell protoplasting (wounding). Gallie's preliminary evidence indicates that phosphorylation of HSP101 regulates its RNA-binding activity. Identification of plant mRNAs that are translationally activated by HSP101 should be forthcoming.

Plant viruses elegantly display several alternative forms of translational control. A.-L.Haenni (Jacques Monod Institute, Paris, France) pondered whether cap-independent initiation, internal ribosome entry, ribosome shunting, frame-shifting, translation of overlapping open reading frames (ORFs) and termination read-through may be solely viral strategies or used by a subset of plant mRNAs. Satellite tobacco necrosis virus (STNV) mRNA lacks both a 5′-cap and a 3′-poly(A) tail. K.Browning (University of Texas, Austin, TX) described analysis of the 3′-translational enhancer of STNV mRNA. An ~100 nucleotide region in the 622 nucleotide 3′-UTR is required for cap-independent initiation in the wheat germ system. This region binds eIFiso4E *in vitro* and in the yeast 3-hybrid RNA–protein binding system. Random mutagenesis supports the prediction that eIFiso4E binds to a stem–loop structure. The 3′-translational enhancer probably functions as a surrogate cap to recruit eIFiso4E or eIF4E and thereby promote initiation. By contrast, M.Skulachev (Moscow State University, Russia) reported that the initiation on a subgenomic mRNA of an *Arabidopsis*-infecting tobamovirus occurs via internal initiation and in the wheat germ system *in vivo* (Ivanov *et al*., 1997). This mode of internal initiation must be distinct from that mediated by the IRES elements of animal enteroviruses, since only a 75 nucleotide region is required and initiation on picornavirus mRNA does not occur in the wheat germ system.

T.Hohn and co-workers (Friedrich Miescher Institute, Basel, Switzerland) discussed the mechanism of non-linear ribosome migration (ribosome shunting) observed for the 5′-capped cauliflower mosaic virus (CaMV) 35S mRNA, as well as Sendai and adeno viruses (Curran and Kolakofsky, 1989; Fütterer and Hohn, 1996; Yueh and Schneider, 1996). Dissection of the *cis*-elements necessary to allow ribosomes to skip over a portion of the 5′-leader revealed three essential components, the 5′-cap and a very short uORF (2–10 codons), immediately preceding a stable hairpin structure. The 400 nucleotide native stem–loop structure of CaMV 35S mRNA can be substituted by Kozak's stem–loop structure of –30 kcal/mol. Toe-printing analyses indicate that the absence of the uORF results in stalling of the pre-initiation complex, which is predicted to impair shunting to the downstream ORF. Ribosome shunting is promoted by the viral-encoded RNA-binding protein, translational activator (TAV). Yeast two-hybrid system and co-immunoprecipitation studies revealed that the C-terminus of TAV interacts with subunit 5 of eIF3 and ribosomal protein L24. As for HSP101, an interaction between TAV and eIF3 could promote association of the 40S pre-initiation complex.

Advances in organellar RNA editing

RNA editing occurs in plant mitochondria and plastids and can restore codons for phylogenetically conserved and functionally important amino acid residues, as well as sequences required for mRNA-processing reactions (Chaudhuri and Maliga, 1997). The plastid genomes of higher plants consist of 120–160 kb circles from which 20–30 C nucleotides are converted to U by a post-transcriptional mechanism. This number is considerably higher in plant mitochondria. How does the editing machinery recognize the Cs to be edited? To address this question, M.Sugiura and co-workers (Nagoya University, Japan) established an *in vitro* editing system prepared from a soluble chloroplast extract of tobacco. Using an elegant assay in which the C to be edited is specifically labeled, Sugiura demonstrated convincingly that this system performs site-specific C to U editing in the *psbL* and *ndhB* transcripts. The 16 nucleotides upstream of the editing site in *psbL* mRNA are critical for editing. Based on UV cross-linking experiments, this region appears to be recognized specifically by a 25 kDa RNA-binding protein. Sugiura proposed that each editing site is recognized by a distinct RNA-binding protein which may recruit a cytidine deaminase. At least eight cytidine deaminase genes have been identified in *Arabidopsis* and the characterization of these enzymes is in progress (J.Weil, University of Strasbourg, France). Using chloroplast transformation and site-directed mutagenesis, R.Bock (University of Freiburg im Breisgau, Germany) identified a 12 nucleotide sequence upstream of two editing sites, eight nucleotides apart, within the *ndhB* mRNA required for editing *in vivo* (Hermann and Bock, 1999). The number of intervening nucleotides, but not their identity, is important for editing. Bock's and Sugiura's data indicate that editing occurs at a fixed distance from a short upstream recognition site.

An open question is whether editing is linked to transcription. Plastid genes of higher plants are transcribed by at least two distinct DNA-dependent RNA polymerases, the eubacterial type (PEP) and phage-type (NEP) polymerases (Hajdukiewicz *et al*., 1997). Using tobacco plants lacking PEP, P.Maliga and co-workers (Rutgers University, New Brunswick, NJ) showed that the editing of NEP read-through transcripts from genes that would normally have been transcribed by PEP, such as *psbL*, *psbE* or *rps14* is absent or greatly reduced. This raises the possibility that editing of specific sites may depend on transcription by a specific RNA polymerase. Comparison of the editing sites in homologous genes present in both plastids and mitochondria revealed that editing in these organelles has evolved independently.

Significant post-transcriptional control in plastids and mitochondria

Chloroplast biogenesis depends on the concerted action of the plastid and nuclear genetic systems. Analysis of mutants of *Chlamydomonas* deficient in photosynthetic activity has revealed the existence of numerous nuclear loci involved in several chloroplast post-transcriptional steps including RNA processing and stability, splicing and translation (Goldschmidt-Clermont, 1998). M.Goldschmidt-Clermont (University of Geneva, Switzerland) described the unusual case of the *psaA* gene of *Chlamydomonas*, which consists of three widely separated exons that are independently transcibed. Transcript maturation requires two steps of *trans*-splicing that involve at least 14 nuclear loci. Three of these loci have been cloned and their products characterized. One of them, Maa2, shows common features with pseudouridine synthases. However, site-directed mutagenesis indicates that this catalytic activity is not required for the function of Maa2 in *psaA trans*-splicing. This factor may thus derive from a pseudouridine synthase that could have been recruited during evolution.

The characterization of two nuclear mutants of *Chlamydomonas* specifically deficient in *psbB* and *psbD* mRNA accumulation was reported by J.-D.Rochaix (University of Geneva, Switzerland). The nuclear genes affected in these mutants encode polypeptides that contain nine tetratricopeptide (TPR) repeats thought to be important for protein–protein interactions. Preliminary results indicate that one of these factors may indeed be part of a multimolecular complex. The target sites of both factors are the 5′-UTR of the corresponding mRNAs. Insertion of a poly(G) sequence into the 5′-UTRs of *psbB* and *psbD* in the mutants restored the stability of these mRNAs, but not their translation, indicating that these factors may also play a role in translation (Nickelsen *et al*., 1999).

Evidence from several groups points to a regulatory feedback connection between photosynthetic activity and chloroplast gene expression which is mediated by phosphorylation and redox control. G.Link (University of Bochum, Germany) described a redox-controlled plastid kinase that appears to regulate the transcription and RNA processing of the *trnK–psbA* co-transcript through phosphorylation of chloroplast σ factors and a site-specific endonuclease, respectively (Liere and Link, 1997). P.Klaff (University of Düsseldorf, Germany) showed that structural alterations of the *psbA* 5′-UTR are induced by changes in Mg^{2+} concentration, which also modulate RNA stability *in vitro*. This may be of physiological relevance since stromal Mg^{2+} levels increase upon illumination.

Ribulose bisphosphate carboxylase (Rubisco), which consists of a nucleus-encoded small subunit and a chloroplast-encoded large subunit, accumulates in bundle sheath cells, but not in mesophyll cells in C4 plants. R.Sawers (University of Oxford, UK) described a nuclear mutation of maize, bundle sheath defective-1 (*bsd2*), which fails to accumulate Rubisco (Brutnell *et al*., 1999). The mutation has no effect on the accumulation of Rubisco mRNAs in bundle sheath cells; however, *rbcL* mRNA accumulates ectopically in mesophyll cells and associates with polysomes. *Bsd2* encodes a polypeptide with a conserved DnaJ-like zinc-finger domain and may chaperone a specific co-translational or post-translational step in Rubisco synthesis.

Relevance of post-transcriptional regulation

The expression of many plant genes is known to be controlled post-transcriptionally (Bailey-Serres and Gallie, 1998; Bailey-Serres, 1999). The relevance of post-transcriptional regulation is elegantly demonstrated by the *Arabidopsis FCA* gene. *FCA* encodes an RNA-binding protein that controls the transition to flowering independent of photoperiod (Macknight *et al*., 1997). P.Sivadon (John Innes Centre, Norwich, UK) reported on the complex post-transcriptional regulation of *FCA*. The *FCA* transcript is alternatively spliced into four transcripts. Only one of these, FCA, encodes a protein that complements the flowering-time defect in *fca-1*. This protein contains two RRM-type RNA-binding motifs and a WW protein interaction domain. Moreover, translation of the FCA transcript produces two polypeptides. Initiation at the first AUG produces a cytosolic isoform, whereas the use of an upstream non-AUG codon results in a nuclear-targeted isoform. An RNA SELEX experiment identified a sequence in the 5′-UTR of the *FCA* mRNA that binds to the RRM domains of FCA. It was subsequently shown that FCA binds its own mRNA, perhaps to regulate initiation of translation from different sites.

Post-transcriptional processes that mediate transgene silencing

Transgene-mediated gene inactivation results in specific inhibition of gene transcription (transcriptional gene silencing, TGS) or specific removal of RNAs from the cytoplasm (post-transcriptional gene silencing, PTGS). TGS and PTGS were previously considered to be unrelated: TGS was assumed to be a consequence of a DNA-mediated *de novo* promoter methylation, whereas PTGS was believed to be RNA-mediated. However, as discussed in Leysin, TGS and PTGS may be based on common processes. O.Voinnet (John Innes Centre, Norwich, UK) presented data that permit the dissection of PTGS into initiation, propagation and maintenance steps. Localized introduction of green fluorescent protein (GFP) gene fragments into plant leaves caused systemic silencing of a previously active GFP transgene (Voinnet *et al*., 1998). Voinnet refined the current model of PTGS. He proposed that silencing is initiated in a small group of cells, possibly by interactions between the introduced DNA and the GFP transgene. In agreement with a model originally proposed for the promoter methylation observed in TGS (reviewed in Meyer, 1995), these interactions lead to methylation of the transgene coding region. As a consequence of methylation, transgene transcription is perturbed and GFP-specific aberrant RNAs (abRNAs) are produced. These abRNAs can then serve as templates for a cellular RNA-directed RNA polymerase (RdRP) that synthesizes complementary RNAs (cRNAs). Formation of GFP mRNA–cRNA duplexes and the degradation of double-stranded regions would then result in PTGS in initiated cells. To ensure systemic silencing (i.e. propagation of PTGS), it is widely assumed that a diffusible silencing signal exists, which is able to spread throughout the entire plant. Indeed, Voinnet and others have shown that in some instances the putative silencing signal can be transmitted from silenced rootstocks to non-silenced target

scions (Voinnet *et al*., 1998; Palauqui and Balzergue, 1999). The spatial pattern of GFP silencing is similar to the movement of infectious virus or viroid RNA. Thus, the putative silencing signal is suggested to contain RNA, probably double-stranded RNA (dsRNA). To maintain PTGS, cells receiving the signal must initiate the degradation process and concurrently amplify the signal.

During the last few months, evidence for the importance of dsRNAs in gene silencing has accumulated. J.Kooter (Vrije University, Amsterdam, The Netherlands) found that chalcone synthase (*Chs*) co-suppression is highly efficient when *Chs* transgene constructs are arranged as inverted repeats (IRs; Stam *et al*., 1998). The heavily methylated IRs were thought to be inactive transcriptionally, but IR-specific transcripts were amplified by RT–PCR. IR transcripts have the potential to fold back into dsRNAs. Thus, these molecules may initiate post-transcriptional silencing of the endogenous *Chs* gene. Alternatively, IRs are assumed to form unusual DNA structures that might interact with homologous DNA sequences, thereby inducing their methylation and affecting *Chs* (trans)gene transcription. Following production of abRNAs (initiation), PTGS would then be transmitted and maintained throughout the plant, as proposed by Voinnet.

Another interesting observation, underlining the essential role of dsRNA and demonstrating the similarity between TGS and PTGS, was documented by F.Mette (Austrian Academy of Sciences, Salzburg, Austria). As put forward by Voinnet, Kooter and others, pairing of homologous DNA may cause DNA methylation, including promoter methylation, whenever TGS is established. To examine whether TGS can be alternatively triggered by a mechanism termed RNA-directed DNA methylation (RdDM), Mette introduced a ^{35}Spro–NOSpro transgene construct into a tobacco line containing NOSpro-driven reporter genes (Mette *et al.*, 1999). In one transformant, *de novo* methylation of all NOSpro sequences and transcriptional inactivation of the target genes were observed. In this plant the ^{35}Spro–NOSpro sequence was arranged as an IR, from which a NOSpro RNA hairpin could be transcribed. Transcription of the NOSpro sequence was necessary for the *trans*-inactivation of NOSpro-driven genes, demonstrating that NOSpro RNA initiated TGS.

The potential of RNA to direct heavy *de novo* methylation of genome-integrated transgenes specifically was shown in M.Wassenegger's laboratory (Fraunhofer IUCT, Martinsried, Germany; Pélissier *et al*., 1999). Transgene sequences displaying homology of as little as 30 bp to the methylation-directing RNA are efficiently targeted by the RdDM mechanism. This demonstrates that the methylation status of a transgene has to be analyzed in great detail to determine whether gene silencing is accompanied by *de novo* methylation. This is an important point, because the absence of DNA methylation has been reported in some examples of PTGS.

The order of processes involved in PTGS was predicted in 1993 (Lindbo *et al.*, 1993), but experimental data are only now starting to confirm these predictions. With the help of RNA inhibitor-chase experiments, F.Meins (Friedrich Miescher Institute, Basel, Switzerland) showed that mRNAs targeted for PTGS are specifically and rapidly degraded in silenced tissue (Holtorf *et al*., 1999). In tissue highly expressing the same gene, the degradation of corresponding mRNAs was normal. A.Hamilton (John Innes Centre, Norwich, UK) demonstrated the accumulation of short RNAs of ~25 nucleotides that were co-linear or complementary to silenced transgene mRNA sequences in plants exhibiting PTGS. The presence of these molecules supports the hypothesis that a cellular RdRP activity may be involved in PTGS. The existence of plant RdRPs is well documented and a cDNA encoding a cellular tomato RdRP has been described (Schiebel *et al*., 1998). The tomato enzyme meets all the requirements for a cRNA-synthesizing enzyme *in vitro*. Recently, a gene-silencing-defective *Neurospora crassa* mutant was isolated and shown to have an inactive RdRP gene due to an insertion mutation (Cogoni and Macino, 1999).

Is PTGS a mechanism involved in normal gene regulation or does it represent a defense mechanism against viruses and foreign DNA? R.B.Flavell (Ceres Inc., Malibu, CA) noted that several features of transgene-induced *Chs* mRNA turnover are found in non-transgenic *Petunia* plants. For example, truncated poly(A)$^+$ *Chs* mRNAs, previously assumed to be characteristic of transgene-mediated *Chs* co-suppression, are present in wild-type cell nuclei (Metzlaff *et al*., 1997). By contrast, N.Al-Kaff (John Innes Centre, Norwich, UK) argued for an involvement of PTGS in virus defense. Upon CaMV infection of oilseed rape three types of homology-dependent transgene responses were observed. Homology between CaMV and transgene promoter sequences led to TGS, homology between the virus and transgene coding regions led to PTGS, whereas transgene expression was enhanced when CaMV and the transgene were unrelated (Al-Kaff *et al*., 1998). These findings indicate that plants endeavor to eliminate viral RNAs and (trans)gene transcripts with homology to an infecting virus. PTGS as a defense mechanism against viruses and foreign genetic elements is also exemplified by the characterization of viral suppressors of gene silencing (2b protein of CMV and HcPro of potyviruses; e.g. Brigneti *et al*., 1998). Hence, further analysis of transgene silencing may reveal basic mechanisms of gene expression and aid the development of genetic engineering strategies.

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