



Commentary

## Novel Plant Activation-Tagging Vectors Designed to Minimize 35S Enhancer-Mediated Gene Silencing

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**Abstract.** Activation tagging is a powerful method of insertional mutagenesis for generating gain-of-function mutations in plants. Current activation-tagging cassettes, based on the 35S enhancer of the cauliflower mosaic virus, have limited utility in genetic backgrounds containing 35S promoter sequences because they may cause homology-dependent gene silencing. We constructed series of novel activation-tagging vectors that do not contain the CaMV 35S enhancer but instead contain multiple tandem copies of an alternative enhancer from cassava vein mosaic virus. For selection, the T-DNAs confer Basta herbicide resistance. Resulting activation-tagging cassettes were introduced into *Arabidopsis thaliana* to demonstrate stable integration of the T-DNA. Vectors described here may be suitable for, but not limited to, activation-tagging projects in genetic backgrounds harboring transgenes driven by the CaMV 35S promoter.

**Key words:** activation tagging, epigenetics, trans-silencing

**Abbreviations:** COP1, constitutive photomorphogenesis1; CsVMV, cassava vein mosaic virus.

### Introduction

Activation tagging is a powerful method for generating gain-of-function mutations by insertional mutagenesis in a variety of plants (Ichikawa et al., 2003; Walden et al., 1994; van der Fits et al., 2001; Jeong et al., 2002; Zubko et al., 2002). A typical activation-tagging cassette contains a strong enhancer element near one end, which may boost the expression of genes within a few kilobase pairs of chromosomal DNA, thus eliciting gain-of-function phenotypes. Activation tagging has become a popular strategy for cutting through the genetic redundancy that limits traditional screens for loss-of-function mutations. With few exceptions (Zuo et al., 2002; Sun et al., 2003), activation-tagging cassettes rely on about 4 tandem copies of the enhancer from the cauliflower mosaic virus (CaMV) 35S promoter (Kakimoto, 1996; Neff et al., 1999; Weigel et al., 2000; Zhao et al., 2001). The 35S enhancer may amplify the expression of nearby endogenous promoters without necessarily causing ectopic expression (Neff et al., 1999). Related activation-

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tagging cassettes have been incorporated into the transposable elements Ac and En-I (Suzuki et al., 2001; Marsch-Martinez et al., 2002).

In principle, activation tagging should be feasible in genetic backgrounds harboring a resident transgene. However, if the resident transgene contains the widely used 35S promoter, the introduction of additional copies of the 35S enhancer may lead to homology-dependent epigenetic gene silencing. To illustrate this point, transcribed inverted repeats of the nopaline synthase promoter and other promoters have been shown to result in methylation and silencing of unlinked homologous copies (Mette et al., 2000; Sijen et al., 2001). Such transcribed inverted repeats can easily arise by chance during T-DNA integration (Mette et al., 1999). Similar processes may underlie the meiotically heritable silencing between pairs of transgene loci carrying the CaMV 35S promoter in tobacco or *Arabidopsis* (Thierry and Vaucheret, 1996; Qin and von Arnim, 2002; Qin et al., 2003). Therefore, when activation tagging is performed with the goal of isolating suppressor mutations of an endogenous 35S-driven transgene, a 35S-based activation-tagging vector is likely to result in false-positive events by directly silencing the resident 35S transgene.

The *Arabidopsis Constitutive Photomorphogenesis1 (COP1)* gene is a repressor of light-regulated development required for the characteristic etiolation response displayed by seedlings germinating in darkness; *COP1* also modulates gene expression and morphogenesis during subsequent vegetative development (Kim et al., 2002). A 35S-driven transgene expressing the COP1 protein as a fusion to beta-glucuronidase (GUS) or green fluorescent protein (GFP) results in the homology-dependent posttranscriptional silencing of the endogenous *COP1* gene, as evident by a characteristic *cop1* mutant phenotype at the adult vegetative stage. Moreover, the 35S:GFP-*COP1* transgene is sensitive to transcriptional trans-silencing by unlinked multimeric T-DNA loci (Qin and von Arnim, 2002; Qin et al., 2003). Trans-silencing is meiotically heritable and is easily visualized by a reversion of the original *cop1*-like cosuppression phenotype. Here we directly demonstrate the potential for false-positive activation-tagging events when a 35S-based activation-tagging cassette is combined with a 35S:GUS-*COP1* transgene. To overcome this obstacle, novel activation-tagging vectors were constructed that are based on the promoter from the pararetrovirus cassava vein mosaic virus (CsVMV). The CsVMV promoter directs strong constitutive gene expression in tobacco and rice (Verdagner et al., 1996, 1998). Importantly, no extended regions of sequence identity between the CaMV and CsVMV promoters exist. Here we describe the construction and initial evaluation in *Arabidopsis thaliana* of activation-tagging vectors based on the CsVMV enhancer as an alternative to CaMV 35S-based vectors.

## Materials and Methods

### *Oligonucleotide-based synthesis of the CsVMV enhancer*

Top and bottom strands of the CsVMV enhancer (base pairs 122-369 of Genbank Accession Number AX088388) were synthesized in the form of 12 overlapping oligonucleotides, ranging in length from 20-50 bases (oligonucleotide sequences available upon request). The 6 salt-free oligonucleotides corresponding to the

upstream portion were combined in a 0.2-mL thin-walled tube (100 pmol each) and heat treated at 94°C for 3 min followed by an immediate ice shock for annealing. Oligos were phosphorylated for 1 h at 37°C by using polynucleotide kinase and then ligated for 3 h at 22°C by directly adding 2 U of T4 DNA ligase in a 20- $\mu$ L reaction. The same procedure was followed for the downstream portion of the enhancer. Two DNA fragments of the expected size were excised from a 1.2% agarose gel, gel purified, and ligated to each other. Part of the product was used as a template for PCR, with the outermost oligonucleotides as primers (XTaq, TaKaRa). PCR conditions were 30 cycles at 94°C for 30 s, 58°C for 40 s, and 72°C for 30 s, followed by an extension at 72°C for 7 min. A resulting 250-bp PCR product was cloned and confirmed by means of DNA sequencing. Next, a tandem tetramer of the CsVMV enhancer was made by first generating 4 different PCR products of the CsVMV enhancer, each containing unique restriction sites at the 5' and 3' ends. The 4 fragments were assembled by standard subcloning in pBluescript to generate pCM4e (details available upon request).

#### *Construction of activation-tagging vectors*

- pAYDH: The tetrameric CsVMV enhancer in pCM4e was subcloned as an *Eco*R I / *Kpn* I fragment to a derivative of the T-DNA vector pCAMBIA-1300 (Genbank AF234296), from which the *lacZ* reporter gene had been deleted by digestion with *Pme* I / *Hind* III and blunt-ending in order to eliminate all possible sequences between the CsVMV enhancer and the right border sequence.
- pAYDB: The Basta-resistance cassette was amplified by PCR from pSKI015 (Weigel et al., 2000) with the primer bar-1 being 5'-TTCCAACATGGTGGC-ATTGAGACCGATGTTCGT-3' and the primer bar-2 being 5'-TTGAATTAA-TTCGCTGAGCCTCGACATGTTGT-3', subcloned to pGEM-T-easy and used to replace the 35S:*hpt* cassette in pAYDH on a *Bst*X I / *Xmn* I fragment.
- pAYDB8: A second tetramer of the CsVMV enhancer was inserted into the *Bam*H I / *Xba* I sites of pAYDB upstream of the 4  $\times$  CsVMV enhancer. Similar procedures were used to insert a tetrameric 35S enhancer from pSKI015 into the *Bam*H I site of pAYDB, downstream of the 4  $\times$  CsVMV, to generate pAYDB44, which was confirmed by means of DNA sequencing.

#### *Synthesis and testing of a CsVMV-enhanced plant expression vector*

The monomeric CsVMV enhancer was blunt-ended and used to replace the double 35S enhancer in the plasmid 35S:*RLUC-YFP* (Xu et al., 2002), thus creating a hybrid regulatory region with a CsVMV enhancer and 90 base pairs of the CaMV 35S transcription start site. Both forward and reverse orientations of the CsVMV enhancer were recovered. Resulting plasmids and controls were tested for transient *Renilla* luciferase expression in *Arabidopsis* rosette leaves after particle-mediated gene delivery (Stacey et al., 1999). Luciferase activity of soluble protein extracts was measured in the presence of 1  $\mu$ mol/L coelenterazine substrate (BioSynth AG, Switzerland) in a Turner Designs TD20/20 luminometer.

### *Arabidopsis transformation*

The pAYD series activation-tagging vectors were introduced into *Agrobacterium* strain GV3101 (pMP90) by electroporation and grown under selection with kanamycin, gentamycin, and rifampicin. An *Agrobacterium* strain containing pSKI015 was kindly provided by Dr Yiji Xia (Danforth Center, St. Louis, MO). *Arabidopsis* wild-type Columbia ecotype was transformed with all activation-tagging vectors following the standard floral dip procedure (Clough and Bent, 1998). The *GUS-COP1* line L4 (Qin and von Arnim, 2002) was transformed with pSKI015 only. Basta was used at 10 mg/L (60% [w/v] phosphinotricin stock, AgrEvo), and hygromycin was used at 25 or 30 mg/L.

### *Isolation of chromosomal sequences flanking the T-DNA*

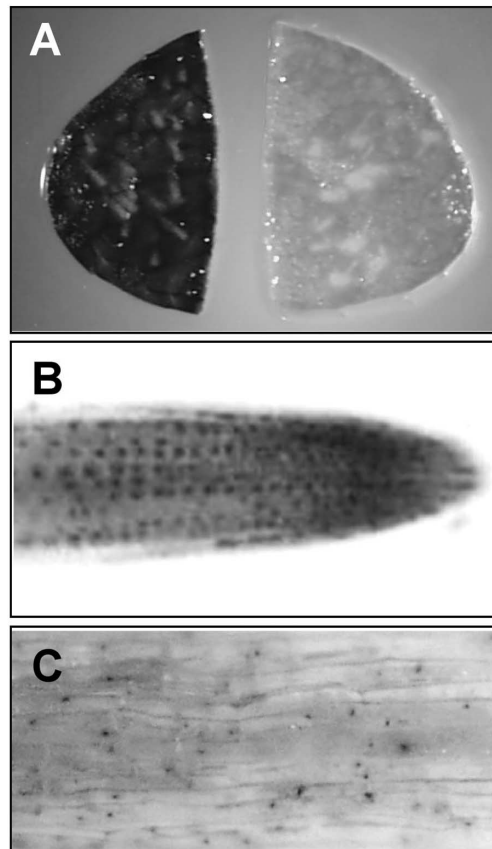
DNA was extracted from *Arabidopsis* flowers or single cotyledons of primary transformants and subjected to thermal asymmetric interlaced-PCR (TAIL-PCR), an iterative procedure using nested primers specific for the T-DNA together with the arbitrary degenerate primer AD2 (Liu et al., 1995). Nested primers were 5'-GGG CAC CAT TCA ACC CGG TCC-3' for round 1, 5'-GTA ACC GAC TTG CTG CCC CGA G-3' for round 2, and 5'-TGC AGG TCA AAC CTT GAC AGT-3' for round 3. These primers amplify sequences adjacent to the T-DNA left border, i.e., opposite the tandem enhancer.

## **Results and Discussion**

### *Transformation of a 35S:GUS-COP1 line with pSKI015*

In an attempt to isolate insertional mutations that disrupt posttranscriptional gene silencing (PTGS) triggered by a 35S:*GUS-COP1* transgene, the single-T-DNA line L4 (Qin et al., 2002) was transformed with the activation-tagging vector pSKI015 (Weigel et al., 2000). Among a small set of primary (T1) transformants recovered from a pilot experiment, one plant (L4-act1) displayed a complete loss of the *cop1*-like cosuppression phenotype characteristic of the parental L4 line (data not shown). For comparison, no such reversion was observed among hundreds of untransformed sibling plants of line L4. L4-act1 also showed intense GUS expression when rosette leaves were histochemically stained, while GUS activity in the parental line L4 was silenced (Figure 1A). The GUS-COP1 protein is known to accumulate in a characteristic subcellular localization pattern, namely nuclear localization in root tip cells and a cytoplasmic inclusion body in other cells (von Arnim and Deng, 1994). Localization of the GUS-COP1 protein was maintained in L4-act1, indicating that the loss of cosuppression was not due to a deletion of the *COP1* portion of the GUS-COP1 transgene (Figure 1B, 1C). Identical results were obtained for a second revertant line, L4-act2 (data not shown). A third transformant, L4-act3, showed a less pronounced reversal of the *cop1*-like cosuppression phenotype.

Although it was plausible that the loss of *COP1* cosuppression was caused by upregulation or gene knockout associated with the activation-tagging cassette, its unexpectedly high frequency led us to suspect other causes. We previously observed that structurally complex T-DNA loci containing the 35S promoter could



*Figure 1.* Reversion of GUS activity in the 35S:GUS-COP1 transgenic line L4-act1 transformed with the activation-tagging vector pSKI015. (A) Left, GUS activity in a rosette leaf of the primary transformant L4-act1. Right, silenced GUS activity in a control parental plant. (B) Nuclear localization of GUS-COP1 in the *Arabidopsis* root tip. (C) Cytoplasmic inclusion bodies of GUS-COP1 in the hypocotyl. The typical subcellular localization of GUS-COP1 argues against a mutation in the *GUS-COP1* transgene.

eliminate cosuppression by a 35S:GFP-COP1 transgene in an epigenetically heritable fashion (Qin et al., 2003). The loss of cosuppression is referred to as trans-silencing, even though the *GFP-COP1* target locus retains substantial transcriptional activity (Qin and von Arnim, 2002). Therefore, we examined whether the activation-tagging cassette in L4-act1 and L4-act2 caused a similar epigenetic transition at the 35S:GUS-COP1 locus. On the basis of the segregation of the Basta resistance gene in the T2 progeny, L4-act1 contained a single pSKI015 transgene locus, and L4-act2 contained 2 loci. T2-generation plants segregating for the activation-tagging cassette were uniformly wild type (Table 1). The lack of *cop1*-like plants suggested that cosuppression by the L4 locus was suppressed by a *cis*-acting event ( $P > 0.95$ ), most likely a heritable epimutation of the L4 locus triggered by the pSKI015 T-DNA. An overall increase in transgene expression is

Table 1. A 35S-based activation-tagging cassette suppresses the cosuppression phenotype associated with a resident 35S:*GUS-COPI* T-DNA.

Transformant*	Number of T-DNA Loci	Phenotypic Segregation Among T2 Plants	
		<i>copI</i> -like (PTGS)	Wild Type (Reverted)
L4-act1	1	0	36
L4-act2	2	0	52
L4-act3	1	18	0
L4 (control)	0	32	0

\*A 35S:*GUS-COPI* transgenic line was retransformed with the activation-tagging vector pSKI015.

typical when PTGS loci are exposed to a trans-silencer (Qin and von Arnim, 2002).

Most known trans-silencer loci are structurally complex. To address this point in the L4-act1 revertant line, we characterized sequences flanking the left border of the pSKI015 T-DNA (Liu et al., 1995). Instead of a contiguous *Arabidopsis* genomic sequence, we recovered 2 T-DNA rearrangements. The first was a junction between the T-DNA left border and the 35S enhancer, and the second was a junction between 2 left border sequences separated by 28 bp of *Arabidopsis* sequence (derived from the *IAA18* gene) and 2 short filler sequences of unknown origin. Regardless of whether a specific T-DNA structure might contribute to the epigenetic effects in the L4-act lines, we suspect that a high ratio of false-positive events must be expected when activation tagging with a 35S enhancer is attempted in a genetic background displaying a 35S-driven transgene phenotype.

#### Construction of new activation-tagging vectors

To build an activation-tagging vector lacking sequence identity with the CaMV 35S promoter, the region of the CsVMV promoter from -75 to -322, which excludes the TATA boxes (CsVMV enhancer), was first synthesized de novo as described in "Materials and Methods." To test whether the CsVMV enhancer is functional in *Arabidopsis*, the duplicate 35S enhancer in the plant expression vector 35S:RLUC-YFP, which contains a *Renilla* luciferase-coding region fused to yellow fluorescent protein, was replaced with a single copy of the CsVMV enhancer. The resulting plasmid retained the TATA box and transcription start site included in the CaMV -90 promoter. Upon transient introduction into *Arabidopsis* rosette leaves, the CsVMV enhancer gave rise to luciferase activity, regardless of its orientation, as did the original duplicated 35S enhancer, while a plasmid lacking an enhancer gave reduced activity (Figure 2). Thus, the CsVMV enhancer appears to function well in *Arabidopsis*.

Next, a tandem tetramer of the enhancer (4 × CsVMV) was inserted in the forward orientation just within the T-DNA right border of a derivative of the vector pCAMBIA-1300. The resulting plasmid, pAYDH, contains a 35S promoter-driven hygromycin resistance gene (35S:*hpt*) as the plant selectable marker gene (Table 2; Figure 3). Because the 35S promoter in the *hpt* gene might again trigger

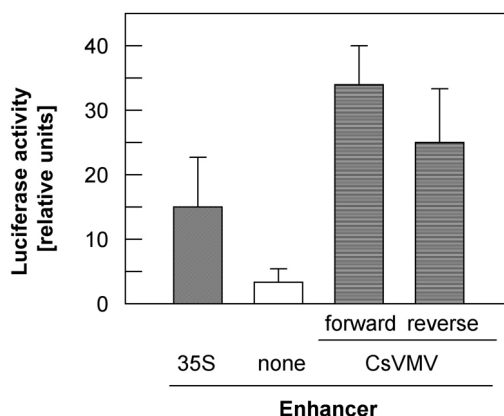


Figure 2. Activity of the CsVMV enhancer. Luciferase expression cassettes carrying the transcription start site from the CaMV 35S promoter as well as the indicated enhancers were introduced into *Arabidopsis* rosette leaves in a transient assay.

Table 2. Plant activation-tagging vectors.

Vector	Enhancer	Plant Selectable Marker	Bacterial Selectable Marker	Reference
pSKI015	4 × CaMV 35S	mas <sup>*</sup> :Basta	Ampicillin	Weigel et al., 2000
pAYDH	4 × CsVMV	CaMV 35S: Hygromycin	Kanamycin	Present work
pAYDB	4 × CsVMV	mas:Basta	Kanamycin	Present work
pAYDB8	8 × CsVMV	mas:Basta	Kanamycin	Present work
pAYDB44	4 × CsVMV and 4 × CaMV 35S	mas:Basta	Kanamycin	Present work

\*mas indicates mannopine synthase promoter from pSKI015 (Genbank Accession AF187951).

homology-dependent gene silencing, the 35S:*hpt* cassette was replaced by the *bar* gene cassette from the classic activation-tagging vector pSKI015, which contains a mannopine synthase (*mas*) promoter and octopine synthase (*ocs*) terminator. The resulting 35S-promoter-free activation-tagging vector was named pAYDB.

Given that the CsVMV enhancer has not been rigorously tested for activity in many plants, we considered that 8 copies of the enhancer might be more effective than the 4 copies present in pAYDB. Therefore, a second tetramer of the CsVMV enhancer was inserted upstream of 4 × CsVMV in pAYDB to generate pAYDB8 (Figure 3). Finally, the tetrameric 35S enhancer from pSKI015 was inserted downstream of the tetrameric CsVMV enhancer in pAYDB to generate the hybrid activation-tagging vector, pAYDB44. This vector should have similar activation efficiency as the original cassette in pSKI015, yet confers kanamycin resistance rather than ampicillin resistance for easier selection in bacteria. The T-DNAs in pAYDB, pAYDB8, and pAYDB44 were introduced into *Arabidopsis thaliana* ecotype Columbia, and primary transgenic plants were recovered successfully on 10 mg/L Basta (data not shown).

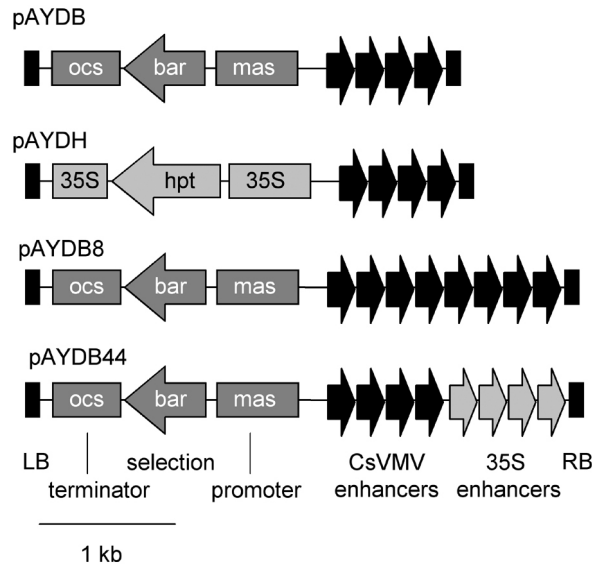


Figure 3. T-DNA structures of new activation-tagging vectors.

To facilitate the identification of chromosomal T-DNA flanking sequences as part of a future activation-tagging project, we tested a set of PCR primers for TAIL-PCR (Liu et al., 1995). Using the primers enumerated in “Methods,” we identified 3 chromosomal insertion sites—base pair 42,565 on BAC F14013 (chromosome 3) for one pSKI015 transgenic plant and base pair 66,332 on BAC T6H22 (chromosome 1) and base pair 96,427 on BAC F7H19 (chromosome 4) for two pAYDB plants. A fourth plant yielded a left border–right border junction.

The sequence identity between the T-DNAs of pAYDB and, for the sake of example, pBIN19 (Genbank U09365), is limited to 729 bp, encompassing 4 sections covering the left and right border, a lacZ sequence, and polylinker sequences. Importantly, there is no sequence identity in their plant coding or promoter sequences and therefore no theoretical basis for mutual epigenetic interference between a resident transgene and the pAYDB activation-tagging cassette. In summary, we have demonstrated that activation tagging may not be practical if the genetic background contains an enhancer sequence also present in the tagging cassette. We have constructed an alternative vector that may be suitable to overcome this problem.

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## References

- Clough SJ and Bent AF (1998) Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. *Plant J* 16: 735-743.
- Ichikawa T, Nakazawa M, Kawashima M, Muto S, Gohda K, Suzuki K, Ishikawa A, Kobayashi H, Yoshizumi T, Tsumoto Y, and others (2003) Sequence database of 1172 T-DNA insertion sites in *Arabidopsis* activation-tagging lines that showed phenotypes in T1 generation. *Plant J* 36: 421-429.
- Jeong DH, An S, Kang HG, Moon S, Han JJ, Park S, Lee HS, An K, and An G (2002) T-DNA insertional mutagenesis for activation tagging in rice. *Plant Physiol* 130: 1636-1644.
- Kakimoto T (1996) CKI1, a histidine kinase homolog implicated in cytokinin signal transduction. *Science* 274: 982-985.
- Kim TH, Kim BH, and von Arnim AG (2002) Repressors of photomorphogenesis. *Int Rev Cytol* 220: 185-223.
- Liu YG, Mitsukawa N, Oosumi T, and Whittier RF (1995) Efficient isolation and mapping of *Arabidopsis thaliana* T-DNA insert junctions by thermal asymmetric interlaced PCR. *Plant J* 8: 457-463.
- Marsch-Martinez N, Greco R, Van Arkel G, Herrera-Estrella L, and Pereira A (2002) Activation tagging using the En-I maize transposon system in *Arabidopsis*. *Plant Physiol* 129: 1544-1556.
- Mette MF, van der Winden J, Matzke MA, and Matzke AJ (1999) Production of aberrant promoter transcripts contributes to methylation and silencing of unlinked homologous promoters in trans. *EMBO J* 18: 241-248.
- Mette MF, Aufsatz W, van der Winden J, Matzke MA, and Matzke AJ (2000) Transcriptional silencing and promoter methylation triggered by double-stranded RNA. *EMBO J* 19: 5194-5201.
- Neff MM, Nguyen SM, Malancharuvi EJ, Fujioka S, Noguchi T, Seto H, Tsubuki M, Honda T, Takatsuto S, Yoshida S, and Chory J (1999) BAS1: A gene regulating brassinosteroid levels and light responsiveness in *Arabidopsis*. *Proc Natl Acad Sci USA* 96: 15316-15123.
- Qin H, Dong Y, and von Arnim AG (2003) Epigenetic interactions between *Arabidopsis* transgenes: characterization in light of transgene integration sites. *Plant Mol Biol* 52: 217-231.
- Qin H and von Arnim AG (2002) Epigenetic history of an *Arabidopsis* trans-silencer locus and a test for relay of trans-silencing activity. *BMC Plant Biol* 2: 11-20.
- Sijen T, Vijn I, Rebocho A, van Blokland R, Roelofs D, Mol JN, and Kooter JM (2001) Transcriptional and posttranscriptional gene silencing are mechanistically related. *Curr Biol* 11: 436-440.
- Stacey MG, Hicks SN, and von Arnim AG (1999) Discrete domains mediate the light-responsive nuclear and cytoplasmic localization of *Arabidopsis* COP1. *Plant Cell* 11: 349-364.
- Sun J, Niu QW, Tarkowski P, Zheng B, Tarkowska D, Sandberg G, Chua NH, and Zuo J (2003) The *Arabidopsis* AtIPT8/PGA22 gene encodes an isopentenyl transferase that is involved in de novo cytokinin biosynthesis. *Plant Physiol* 131: 167-76.
- Suzuki Y, Uemura S, Saito Y, Murofushi N, Schmitz G, Theres K, and Yamaguchi I (2001) A novel transposon tagging element for obtaining gain-of-function mutants based on a self-stabilizing Ac derivative. *Plant Mol Biol* 45: 123-131.
- Thierry D and Vaucheret H (1996) Sequence homology requirements for transcriptional si-

- lencing of 35S transgenes and post-transcriptional silencing of nitrite reductase (trans)genes by the tobacco 271 locus. *Plant Mol Biol* 32: 1075-1083.
- van der Fits L, Hilliou F, and Memelink J (2001) T-DNA activation tagging as a tool to isolate regulators of a metabolic pathway from a genetically non-tractable plant species. *Transgenic Res* 10: 513-521.
- Verdaguer B, de Kochko A, Beachy RN, and Fauquet C (1996) Isolation and expression in transgenic tobacco and rice plants of the cassava vein mosaic virus (CVMV) promoter. *Plant Mol Biol* 31: 1129-1139.
- Verdaguer B, de Kochko A, Fux CI, Beachy RN, and Fauquet C (1998) Functional organization of the cassava vein mosaic (CsVMV) promoter. *Plant Mol Biol* 37: 1055-1067.
- von Arnim AG and Deng X-W (1994) Light inactivation of *Arabidopsis* photomorphogenic repressor COP1 involves a cell type specific modulation of its nucleocytoplasmic partitioning. *Cell* 79: 1035-1045.
- Walden R, Fritze K, Hayashi H, Miklashevichs E, Harling H, and Schell J (1994) Activation tagging: a means of isolating genes implicated as playing a role in plant growth and development. *Plant Mol Biol* 26: 1521-1528.
- Weigel D, Ahn JH, Blazquez MA, Borevitz JO, Christensen SK, Fankhauser C, Ferrandiz C, Kardailsky I, Malancharuvil EJ, Neff MM, and others (2000) Activation tagging in *Arabidopsis*. *Plant Physiol* 122: 1003-1013.
- Xu Y, Kanauchi A, von Arnim AG, Piston DW, and Johnson CH (2002) Bioluminescence resonance energy transfer: monitoring protein-protein interactions in living cells. In: Mariott AG and Parker I (eds), *Methods in Enzymology; Biophotonics*, vol 360, pp. 289-301.
- Zhao Y, Christensen SK, Fankhauser C, Cashman JR, Cohen JD, Weigel D, and Chory J (2001) A role for flavin monooxygenase-like enzymes in auxin biosynthesis. *Science* 291: 306-309.
- Zubko E, Adams CJ, Machaekova I, Malbeck J, Scollan C, and Meyer P (2002) Activation tagging identifies a gene from *Petunia hybrida* responsible for the production of active cytokinins in plants. *Plant J* 29: 797-808.
- Zuo J, Niu Q-W, Frugis G, and Chua N-H (2002) The WUSCHEL gene promotes vegetative-to-embryonic transition in *Arabidopsis*. *Plant J* 30: 349-359.