

GENOMICS ARTICLE

Gene Traps: Tools for Plant Development and Genomics

Patricia S. Springer¹

Department of Botany and Plant Sciences, University of California, Riverside, California 92521-0124

INTRODUCTION

Classical genetic approaches to gene identification rely on disruption of a gene leading to a recognizable phenotype. This approach continues to be an extremely successful one, yielding mutations that result in overt phenotypes reflecting the function of the corresponding gene. Not all genes, however, can be uncovered by mutagenesis for two main reasons. First, many genes are functionally redundant, sharing overlapping functions with other genes that may or may not be related at the sequence level. Mutation of a functionally redundant gene is not likely to lead to an easily recognizable phenotype, because one or more other family members can provide the same function. Analysis of systematic gene knockouts has revealed that a significant percentage of yeast genes have no obvious phenotype when disrupted, despite testing under a wide range of growth conditions (Ross-MacDonald et al., 1999). Therefore, it is likely that disruption of many plant genes will not result in an easily identifiable phenotype. Second, many genes function at multiple stages of development. Mutations in these genes may lead to early lethality or may be highly pleiotropic, which can mask the role of a gene in a specific pathway.

A second common method of gene identification is based on expression patterns. In this case, genes that are expressed in spatially, temporally, or conditionally restricted patterns are isolated, traditionally via some type of differential screening approach. Recently, the differential mRNA display technique (Reuber and Ausubel, 1995) has increased the sensitivity of this approach. Additionally, recent developments with microarray and gene chip technologies allow the expression profiles of many genes to be analyzed (Richmond and Somerville, 2000). Nevertheless, these approaches are ultimately limited by the source of tissue used to isolate the RNA probe. Genes that are expressed transiently, at low levels, or in a small number of cells are unlikely to be identified.

In recent years, techniques to identify genes have been

developed that utilize random integration of reporter gene constructs. This approach has been called enhancer detection in *Drosophila* (reviewed in Bellen, 1999) and has also proven to be an extremely powerful tool in mouse developmental biology. In this review, I describe how enhancer detection systems have been adapted to plant biology so as to add to the arsenal of gene identification techniques available to the plant biology community.

Several different types of "trapping" systems have been developed. The major difference among such systems lies in the reporter gene construct that is used. All these systems have been designed for gene identification, and the generic term "gene trap" is therefore used to refer to them collectively, regardless of the specific reporter gene construct.

BACKGROUND

A system that allows gene activity to be monitored by creating gene fusions with a reporter gene was first used in bacterial genetics >20 years ago (Casadaban and Cohen, 1979). Random insertions of a *lacZ* reporter gene into the *Escherichia coli* chromosome were generated. These created gene fusions that could be used to monitor the expression of individual genes. In this way, genes could be identified based on their pattern of expression over time or under a variety of conditions in the absence of a mutant phenotype or any sequence information. Because the sequence of the inserted DNA was known, it provided a "tag" for easy isolation of the chromosomal gene. This approach has been modified for use in a number of species and has been extensively exploited in *Drosophila* and mouse genetics (reviewed in Bellen, 1999).

Reporter genes can be used to construct three basic types of gene trap: enhancer trap, promoter trap, and gene trap (Figure 1). Each type is able to respond to *cis*-acting regulatory sequences at the site of insertion. In an enhancer trap (Figure 1B), the reporter gene is fused to a minimal promoter, typically containing a TATA box and transcription start site, that is unable to drive reporter gene expression

¹To whom correspondence should be addressed. E-mail patricia.springer@ucr.edu; fax 909-787-4437.

alone but can be activated by neighboring enhancer elements. Promoter traps and gene traps contain a promoterless reporter gene so that expression can occur only when the insertion is within a transcriptional unit and in the correct orientation (Figures 1C and 1D). Expression of a promoter trap reporter gene requires that it be inserted into an exon, leading to a transcriptional fusion (Figure 1C). In contrast, gene trap constructs contain one or more splice acceptor sequences preceding the reporter gene (Figure 1D), which allow expression if insertion occurs in an intron. Splicing from the splice donor sites in the chromosomal gene to the splice acceptor sites in the reporter gene results in fusion of upstream exon sequences to the reporter gene. In addition to transcriptional fusions, promoter trap and gene trap inser-

tions can also create translational fusions, which may provide information about protein localization.

Each type of reporter gene construct has its own advantages. Because enhancer traps do not have the same constraints on expression as promoter and gene traps, which must insert within a gene and in the correct orientation, enhancer trap insertions lead to a higher frequency of reporter gene expression. However, expressed promoter or gene traps are more likely to cause gene disruption than are expressed enhancer traps. Also, because enhancers can activate gene expression at considerable distances, the genes controlling reporter gene expression may be more easily identified in promoter or gene trap insertions than in enhancer trap insertions.

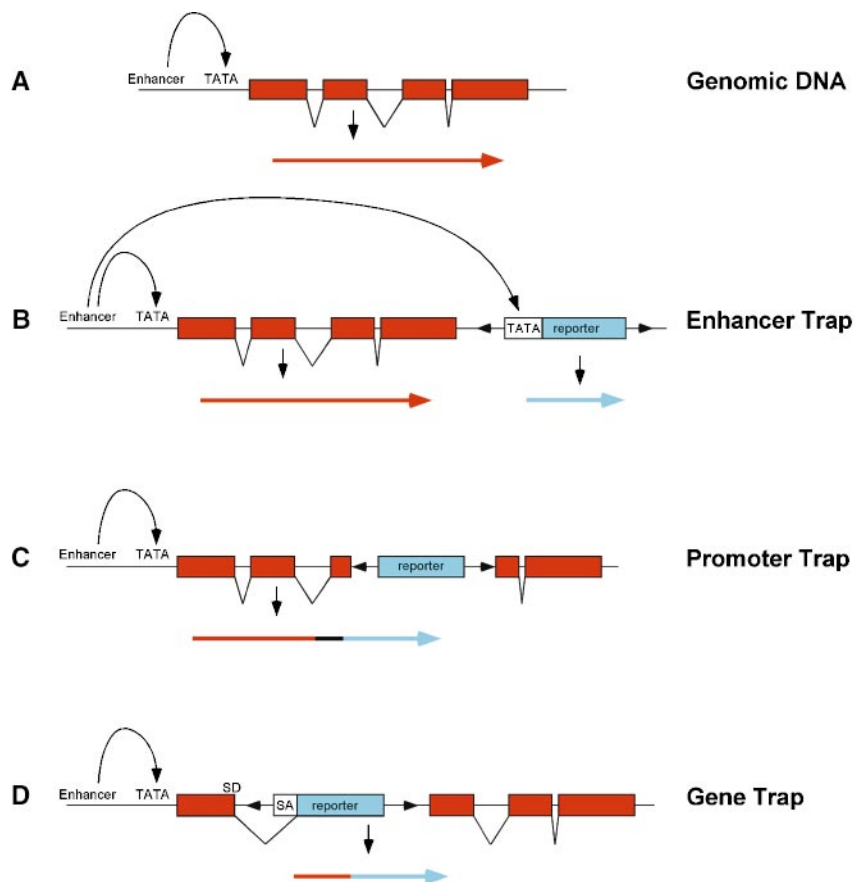


Figure 1. Structure of Enhancer, Gene, and Promoter Trap Vectors.

(A) A generic chromosomal gene with exons (boxes) and introns (lines).

(B) Enhancer trap construct. The minimal promoter of the reporter gene (TATA) is activated by a chromosomal enhancer element, resulting in expression of the reporter gene.

(C) Promoter trap construct. The promoterless reporter gene can be expressed when insertion occurs in an exon so as to result in a transcriptional fusion.

(D) Gene trap construct. The promoterless reporter gene contains splice acceptor (SA) sequences. Expression of the reporter gene occurs upon its insertion into an intron. Splicing from the chromosomal splice donor (SD) site to the SA sequence results in creation of a transcriptional fusion.

Arrows in each panel represent the transcripts that are produced as a consequence of insertion.

Why Use Gene Traps?

Gene traps provide a powerful tool for gene identification. Genes are identified based on reporter gene expression; therefore, a mutant phenotype is not required. This advantage allows identification of two classes of genes that are not easily amenable to classic genetic analysis: functionally redundant genes and genes that have functions at multiple developmental stages. The simultaneous identification of mutations in two redundant genes has in fact only rarely been attained (e.g., Wilhelmi and Preuss, 1996; Aida et al., 1997), and most mutations in redundant genes have thus gone undetected by traditional means. In addition, disruption of a gene might result in a subtle phenotype that could go undetected. However, if a gene trap insertion disrupts a gene, then the expression pattern might suggest the type of phenotype to look for and may lead to observation of phenotypes that could otherwise be overlooked.

On the other hand, a mutation in a gene that is required at multiple stages of development is likely to result in lethality, whereas pleiotropic genes may result in complex phenotypes that are difficult to approach experimentally. Gene traps allow genes to be identified based solely on expression pattern, so that loss-of-function mutations are not mandatory. Although a gene trap insertion may disrupt gene function, the disruption per se is not necessary for gene identification. Therefore, functionally redundant genes whose expression patterns meet screening criteria can be identified in the absence of an easily recognizable phenotype. Moreover, essential genes can be identified based on reporter gene expression, moreover, in viable heterozygotes, even if disruption causes lethality. Likewise, genes that have roles at multiple developmental stages can be identified based on expression, even if mutations occur within pleiotropic genes.

To use gene traps, a collection of individuals is generated that contains a reporter gene integrated randomly into the genome. Various strategies for obtaining efficient reporter gene expression have been utilized (see below). Each insertion is maintained as a separate line, which can subsequently be screened for reporter gene expression. Screens can be designed to identify genes that are expressed in specific cells or tissues, at specific developmental stages, or in response to an environmental stimulus.

Gene Traps in Flies and Mice

An enhancer trap system was first described in *Drosophila* 13 years ago (O'Kane and Gehring, 1987). The authors used *P*-element-mediated transformation to introduce the reporter *lacZ* gene into the genome. The *lacZ* gene was driven by the weak, constitutive *P*-element promoter, so that its expression could not be detected by staining for β -galactosidase activity. Insertions of this construct into the genome conferred many different cell- and tissue-specific *lacZ* ex-

pression patterns, demonstrating that *cis*-acting regulatory elements could be detected using this approach. Second-generation systems used transposition rather than transformation to recover fly lines containing *P*-element insertions and a plasmid rescue cassette for rapid isolation of flanking genomic DNA (Bellen et al., 1989; Bier et al., 1989; Grossniklaus et al., 1989; Wilson et al., 1989).

At about the same time, traps were described in mice (Allen et al., 1988; Kothary et al., 1988). Initially, both enhancer and promoter traps were tested (Gossler et al., 1989). Because enhancers can act over large distances, it has been argued that the genes associated with an enhancer might be more difficult to identify with enhancer traps than promoter or gene traps (Skarnes, 1990). This argument might especially hold for organisms with large genomes and low gene density. For this reason, work in mouse has tended to use promoter or gene trap vectors. Gene trap constructs are typically introduced into totipotent embryonic stem cells by using a retroviral vector. Embryonic stem cells that subsequently test positive for retroviral integration are subsequently transferred to the mouse germline. The gene trap vector commonly used in mouse contains a *lacZ* reporter gene with one or more splice acceptor (SA) sequences (reviewed in Rossant and Hopkins, 1992; Brennan and Skarnes, 1999). If insertion occurs in an intron, then splicing will create a transcriptional fusion between the adjacent exon sequence and *lacZ*. In practice, use of SA sequences increases the frequency of insertions that result in reporter gene expression by 10- to 100-fold (Gossler et al., 1989; Skarnes, 1993).

GENE TRAPS IN PLANTS

Summary of Plant Systems

The first-generation gene trap systems in plants were designed to determine how frequently T-DNA insertions integrated into genes. Early experiments were performed by transforming tobacco protoplasts with a T-DNA containing a promoterless antibiotic resistance gene adjacent to one border (André et al., 1986; Teeri et al., 1986). Recovery of a transformed plant relied on the generation of a gene fusion that led to expression of the antibiotic resistance gene. Therefore, this approach was limited to detection of gene fusions that were expressed in regenerating tissues. Further modifications of the experimental approach incorporated a second selectable marker in the T-DNA, so that transformed plants could be regenerated and subsequently screened for expression of the promoterless antibiotic resistance gene (Koncz et al., 1989; Herman et al., 1990). The next step was inclusion of the β -glucuronidase (*gusA* or *uidA*) reporter gene that could be easily visualized by histochemical staining (Fobert et al., 1991; Kertbundit et al., 1991; Topping et al.,

1991). This advance allowed spatial and temporal expression patterns to be visualized. Constructs that contained either a promoterless *gusA* gene or a *gusA* gene driven by a minimal promoter were used. More recently, transposable elements have been used to deliver enhancer or gene traps into plant genomes (Fedoroff and Smith, 1993; Klimyuk et al., 1995; Sundaresan et al., 1995).

Choice of Insertion Vehicle

The two main alternatives for delivery of enhancer and gene trap reporters into plant genomes are T-DNAs and transposable elements. There are advantages and disadvantages to each, which are summarized below.

T-DNA

T-DNA-mediated transformation is a common way to generate transgenic plants. In plants in which transformation efficiency is reasonably high, the use of T-DNAs allows large collections of independent insertions to be quickly generated. Because T-DNAs are not known to insert with site specificity, it should be possible to saturate the genome with T-DNA insertions (Azpiroz-Leehan and Feldmann, 1997). In fact, large T-DNA collections have been generated in Arabidopsis (Feldmann and Marks, 1987; Bouchez et al., 1993; Campisi et al., 1999; Krysan et al., 1999; Weigel et al., 2000). Systematic efforts are now underway to use these collections for "reverse genetic" screens to identify insertions in any cloned gene (McKinney et al., 1995; Winkler et al., 1998; Krysan et al., 1999). The use of T-DNAs for insertional mutagenesis is limited, however, to those plant species that can be easily transformed by T-DNA.

Significantly, multiple T-DNA insertions often occur in a single plant, both in multiple copies per locus and in multiple loci (Bechtold et al., 1993; Lindsey et al., 1993). This multiplicity is a potential problem when delivering enhancer or gene trap reporter genes, because multiple insertions may complicate interpretation of expression patterns. Additionally, T-DNA insertion events can often be complex, generating T-DNA repeats in direct or inverted orientations, with occasional rearrangements of adjacent chromosomal DNA (Ohba et al., 1995; Nacry et al., 1998; Laufs et al., 1999). These complexities may result in reporter gene expression from promoters introduced along with the T-DNA rather than from chromosomal promoters. Complex insertions may also complicate subsequent molecular analyses, making it difficult to isolate the chromosomal genes driving reporter gene expression. Finally, genomic T-DNA insertions are generally stable, so that remobilization is not readily possible, as it is for transposable elements (see below).

T-DNA vectors have been used by a number of different groups to deliver gene trap constructs into plants. En-

hancer, promoter, and gene trap reporter genes have been used, and expression of reporter genes has been efficient with the reporter gene positioned at either the left (Lindsey et al., 1993) or right (Campisi et al., 1999) border of the T-DNA.

Transposable Elements

Transposable elements are routinely used to perform insertional mutagenesis. In species that do not have active, well-characterized transposable element systems, heterologous elements can be utilized (reviewed in Osborne et al., 1991). In this case, the elements are introduced into the plant genome by T-DNA-mediated transformation and subsequently mobilized. Insertional mutagenesis with transposable elements offers several advantages over T-DNAs. Although integrated transposable elements in the absence of transposase (see below), like integrated T-DNAs, are stable, transposable element insertions can be selectively destabilized upon expression of transposase. In this way, remobilization can generate germinal revertants so as to verify that a phenotype is indeed caused by insertion of the transposon. Somatic revertant sectors can also be generated for clonal analysis, which is useful for studying the cell-autonomous nature of the given gene product. Finally, because some eukaryotic elements preferentially transpose to closely linked locations (Greenblatt, 1984), derivative alleles can be generated by remobilizing an element (Das and Martienssen, 1995).

The maize *Ac/Ds* and *En/Spm* transposable elements have been developed for use in heterologous species. The behavior of these elements has been extensively studied, and they have been modified for efficient transposition in plants such as tobacco, tomato, and Arabidopsis (reviewed in Osborne and Baker, 1995). To date, only the *Ac/Ds* system has been used for enhancer or gene trap systems, although it is possible that other elements will also prove useful for this purpose. An important consideration when choosing a transposable element system is copy number. The *Ac/Ds* system has been chosen due to its low copy number (Bancroft et al., 1992). Elements that have a tendency to amplify, such as *En/Spm* (Aarts et al., 1995), potentially complicate interpretation of reporter gene expression patterns.

The *Ac/Ds* transposable element system is a two-component system with autonomous (*Ac*) and nonautonomous (*Ds*) components. The *Ac* element encodes a transposase that binds to the terminal inverted repeat ends of both *Ac* and *Ds* elements, catalyzing their transposition to new locations in the genome. *Ds* elements are most often derivatives of *Ac* that have lost the ability to produce a transposase but retain the terminal inverted repeats. The *Ac* transposase, when produced in *trans*, is able to recognize the ends of *Ds* elements and catalyze their movement to new chromosomal locations. The use of a two-component system allows for

stable insertions to be generated, because the autonomous element can be segregated away from the insertion. Such insertions, moreover, can subsequently be remobilized by introducing the transposase via genetic crosses. Remobilization can allow somatic and germinal revertants and derivative alleles to be isolated.

As in maize, *Ac/Ds* elements demonstrate a marked preference for transposition to genetically linked locations in Arabidopsis and other plants (Greenblatt, 1984; Dooner and Belachew, 1989; Jones et al., 1990; Dooner et al., 1991; Osborne et al., 1991; Bancroft and Dean, 1993; Carroll et al., 1995; Machida et al., 1997). Although this feature can be advantageous at times, it may be problematic when the goal is to generate random insertions throughout the genome. Schemes have been designed to enrich for transposition events that are not linked to the donor locus (Sundaresan et al., 1995).

Choice of Reporter Gene

β -Glucuronidase

The bacterial *gusA* (*uidA*) gene is a commonly used reporter gene in plants. The GUS protein is quite stable, and it retains activity when it is fused to other proteins (Jefferson et al., 1987). GUS activity can be detected by histochemical staining using a variety of substrates that are commercially available. Unfortunately, most of the substrates are expensive, and the histochemical stains are destructive, so that GUS assays cannot be performed on live tissue. Detection of GUS activity is very sensitive (Jefferson et al., 1987; Lindsey et al., 1993), however, and activity can even be detected in single cells. The cost of GUS substrate prohibits its use for large plants or for large-scale screens on many plants. Because Arabidopsis plants are quite small, however, individuals can be stained in small volumes of substrate solution.

Green Fluorescent Protein

The jellyfish green fluorescent protein (GFP) has recently been used as a reporter gene in plants, although modifications were required to obtain GFP proteins that fluoresce efficiently in Arabidopsis (Haseloff and Amos, 1995; Siemering et al., 1996; Haseloff et al., 1997). Because GFP is fluorescent, it can be directly detected by illumination; no substrate is required, and detection is therefore relatively inexpensive, provided the appropriate light source is available. Detection of GFP activity is also nondestructive, can be performed in live cells, and can be monitored over time. Recent modifications have improved the sensitivity of GFP (Haseloff et al., 1997), making it a useful reporter for gene trap systems.

Other Options

Other reporter genes might also be useful. *Lc* is a member of the maize *R* gene family of Myc-like transcription factors, regulates anthocyanin biosynthesis, and is particularly promising as a reporter gene. Expression of *Lc* in heterologous plants leads to anthocyanin accumulation (Lloyd et al., 1992; Goldsbrough et al., 1996). This system is thus advantageous because detection does not require expensive substrate and is nondestructive. This type of visual reporter is especially attractive for screens of large, field-grown plants, for which illumination for GFP detection might be impractical. However, expression of high levels of the *Lc*-encoded transcription factor may have phenotypic effects. For example, high levels of expression of *Lc* in Arabidopsis lead to an increase in the number of trichomes formed.

Transposable Elements: Tagging Strategies

When transposable elements are used for insertional mutagenesis, careful consideration should be given to the strategy used to recover new insertions. The two most common types of systems, relying either on selection for excision or on selection for transposition, are described below, using the *Ac/Ds* system as an example. In both approaches, *Ac* and *Ds* elements are introduced into the genome via T-DNA-mediated transformation, and single-locus T-DNA insertions are identified. Typically, the *Ac* and *Ds* elements are introduced on separate T-DNA constructs, but they can also be introduced on the same construct if a counterselectable marker (see below) is used. A single T-DNA approach might be advantageous in plants for which crossing is laborious. Only a few transgenic plants need to be generated, because homozygous transformants provide the starting parental material. Most often, the *Ac* element is modified by removal of one of the terminal inverted repeat ends so that it is unable to transpose and simply provides a source of transposase. Mutagenesis is initiated by intercrossing homozygous *Ac* and *Ds* parents. During development of an F₁ plant that is heterozygous for both elements, the *Ds* element will transpose. F₁ plants are selfed to recover F₂ plants in which the *Ds* element has transposed and segregated away from *Ac*. Recovery of plants that no longer have an autonomous element (*Ac*) allows the new *Ds* insertions to be stabilized. The *Ac* element can be either selected against or screened out (see below).

Selection for Excision

Transposon mutagenesis is initiated from a donor *Ds* element previously introduced into the genome by transformation. In a system that selects for excision, as shown in Figure 2A, the donor *Ds* element, carrying a ubiquitously

expressed antibiotic resistance gene, arrives within a T-DNA construct to the plant genome by *Agrobacterium*-mediated transformation. Within the T-DNA, the *Ds* element resides in the untranslated leader of a second ubiquitously expressed antibiotic resistance gene. Transformed plants are therefore sensitive to the second antibiotic because the presence of the *Ds* element prevents expression of the resistance gene. Upon mobilization, the *Ds* element excises, resulting in expression of the second antibiotic resistance gene (Jones et al., 1989). Seedlings that are resistant to both antibiotics therefore reflect a transposition event wherein the *Ds* element has excised from its T-DNA location and reinserted in another chromosomal location (Figure 2A). Because excision of a *Ds* element is not always followed by reinsertion (Bancroft and Dean, 1993; Long et al., 1993), it is important to select for the presence of the *Ds* element. A host of different antibiotic resistance genes are available for use (reviewed in Osborne and Baker, 1995). Resistance genes

against herbicides that can be applied to soil-grown seedlings have also been used (DeBlock et al., 1987; Tissier et al., 1999), thereby reducing the labor involved in the screening process.

In general, selection for excision is very effective, although not always ideal. One problem is that excision events occurring late in the development of the F_1 plants (after the male and female lineages have diverged) are present in the male but not the female gametes, or vice versa. When this situation occurs, an F_2 plant that is resistant to both antibiotics does not necessarily contain a transposed element (Figure 2A, class 3; Long et al., 1993). In addition, because >50% of transpositions occur to linked locations (Greenblatt, 1984; Dooner and Belachew, 1989; Jones et al., 1990; Dooner et al., 1991; Osborne et al., 1991; Bancroft and Dean, 1993; Carroll et al., 1995; Machida et al., 1997), many of the transposed elements recovered using this scheme are linked to the donor locus (Figure 2A, class 1). To obtain broad cover-

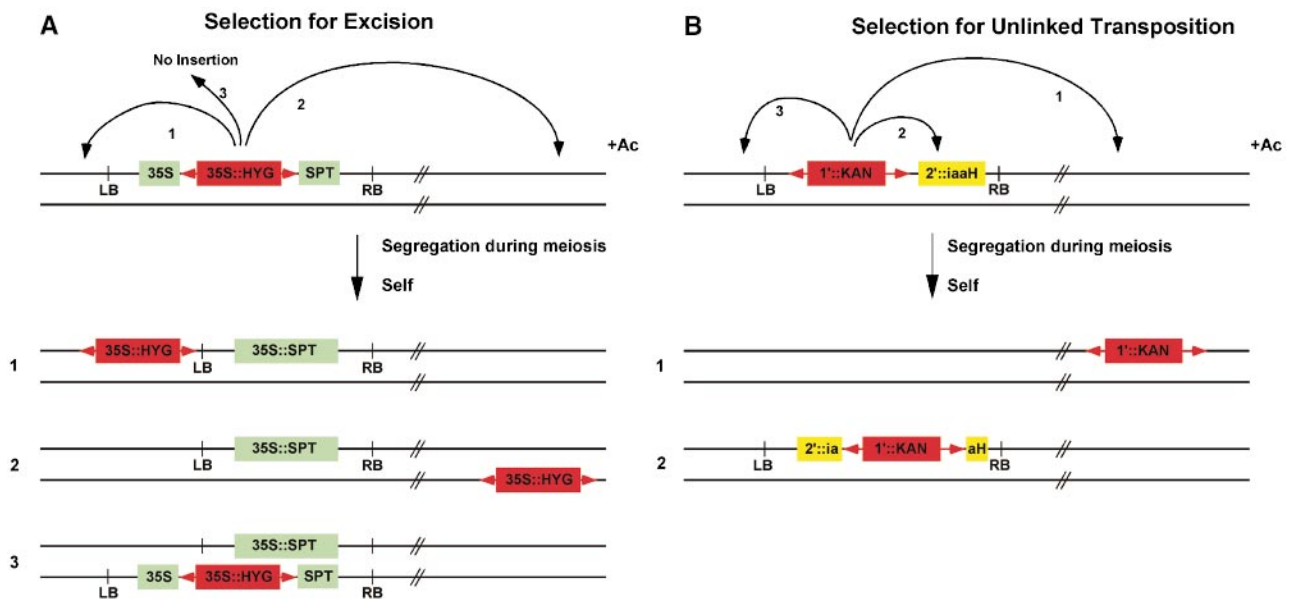


Figure 2. Transposable Element Tagging Strategies.

(A) Selection for excision. The donor *Ds* element, shown in red, carries an antibiotic resistance gene, *35S::HYG*, and is inserted in the untranslated leader of a second antibiotic resistance gene, *35S::SPT*, which resides on a T-DNA. In the presence of *Ac*, *Ds* excises, restoring the *35S::SPT* gene and resulting in resistance to streptomycin. F_2 plants that are resistant to streptomycin and hygromycin contain a *Ds* element that has excised from its original location in the T-DNA. Transposition to linked locations (class 1) or unlinked locations (class 2) is recovered. Class 3 plants, which do not contain a transposed element, are recovered when the *Ds* element has excised in the egg but not sperm (or vice versa).

(B) Selection for transposition. The donor *Ds* element, shown in red, carries an antibiotic resistance gene, *1'::KAN*, and is adjacent to a counterselectable marker gene, *2'::iaaH*, within a T-DNA. In the presence of *Ac*, the *Ds* element excises. F_2 plants that are resistant to both kanamycin and naphthalene acetamide (NAM) carry a *Ds* element but not an *iaaH* gene. This selection enriches for recovery of unlinked transpositions. Linked transpositions (class 3) are not recovered due to counterselection against the *iaaH* gene. Linked transpositions that inactivate the *iaaH* gene (class 2) are recovered because plants are NAM resistant.

LB, left border of T-DNA; RB, right border of T-DNA.

age throughout the genome, many transposition events must therefore be isolated.

When the goal is to target a gene that is linked to the donor locus, this system works extremely well. A number of genes have been cloned in Arabidopsis and other plants based on this "directed tagging" approach (e.g., Jones et al., 1994), and efforts are under way to systematically map donor T-DNA loci for use in directed tagging of linked genes (Smith et al., 1996; Long et al., 1997; <http://www.jic.bbsrc.ac.uk/staff/caroline-dean/dslaunch.htm>).

Selection for Transposition

To select for unlinked transposition events (Figure 2B), the donor *Ds* element, carrying a ubiquitously expressed antibiotic resistance gene, is introduced into plants via T-DNA transformation. A ubiquitously expressed counterselectable marker gene, whose presence can be selected against, is also present on the T-DNA. F₂ seedlings that are resistant to both positive and negative selective agents will therefore contain a *Ds* element that has transposed to a new location and segregated away from the T-DNA. Using this system, linked transposition events are selected against, thereby enriching for insertions that are not linked to the donor. This scheme allows for efficient recovery of insertions throughout the genome (Sundaresan et al., 1995; Parinov et al., 1999). Counterselectable marker genes are typically conditional, acting by conversion of an innocuous compound into one that is toxic to plant cells. Several different counterselectable marker genes have been used in plants, including *indoleacetamide hydrolase* (*iaaH*; Klee et al., 1987), *cytosine deaminase* (*codA*; Stougaard, 1993), and *cytochrome P450_{SU1}* (*SU1*; O'Keefe et al., 1994). *iaaH* confers sensitivity to naphthalene acetamide (NAM); *codA* confers sensitivity to 5-fluorocytosine; and *SU1* confers sensitivity to the sulfonylurea proherbicide R7402.

One problem with this system is that linked transpositions within the donor T-DNA may disrupt the counterselectable marker. This type of insertion leads to the recovery of double-resistant seedlings that do not have a useful transposed *Ds* element (Figure 2B, class 2). In practice, this occurs in 5 to 15% of double-resistant seedlings (Sundaresan et al., 1995; Parinov et al., 1999). This problem might be avoided by including two copies of a counterselectable marker within the T-DNA; however, this might also lead to cosuppression, which could inactivate the counterselectable marker.

Although *Ds* elements exhibit no known insertion site specificity, transposition of unlinked *Ds* elements does not result in an entirely random distribution. Parinov et al. (1999) have determined the map location of 356 *Ds* insertions. They amplified sequences flanking the insertions and determined map positions using the characterized Arabidopsis genomic sequence. *Ds* elements were inserted on all five Arabidopsis chromosomes, with a higher frequency of inser-

tions found on chromosomes 2 and 4 (likely reflecting the greater amount of sequence information available for those two chromosomes at the time of the study; Lin et al., 1999; Mayer et al., 1999). However, apparent transposition "hot spots" were observed. A major bias for insertion near the nucleolus organizer regions on chromosomes 2 and 4 was seen, and several other regions also appeared to contain more insertions than would be expected for random distribution (Parinov et al., 1999). Additionally, preferential insertion near the 5' ends of genes was observed (Parinov et al., 1999). Preferential insertion into the 5' ends of genes has also been observed for other transposable elements, including *Drosophila P* elements, and may reflect the ability of elements to insert into "open" chromatin (Spradling et al., 1995).

Cold Spring Harbor Gene Trap System

A gene trap system that uses selection for transposition was developed at Cold Spring Harbor Laboratory (Sundaresan et al., 1995). This system uses the *Ac/Ds* transposable elements and a positive/negative selection for transposition. Both enhancer trap and gene trap reporter gene constructs have been developed. The transposase in the immobilized *Ac* element is driven by the cauliflower mosaic virus (CaMV) 35S promoter. The *Ac* is introduced into plants by T-DNA-mediated transformation, and an *iaaH* gene, driven by the ubiquitously expressed 2' promoter is also present on the *Ac* T-DNA. The *Ds* elements were constructed by replacing sequences of the maize *Ac* element with an *NPTII* gene driven by the ubiquitously expressed 1' promoter and a *GUS* reporter gene. The *GUS* gene lies at the 3' end of the element, relative to the *Ac* sequence (GenBank accession number for the *Ac* sequence is X05424). The *GUS* gene in the enhancer trap element (*DsE*) is fused to a minimal promoter from the CaMV 35S gene. This region of the promoter has no detectable activity unless chromosomal enhancer elements are nearby (Benfey et al., 1989). The *GUS* gene in the gene trap element (*DsG*) is promoterless and contains three SA sites in each of three reading frames, fused upstream of the initial ATG codon. This construct allows *GUS* expression via transcriptional and translational fusion if the *DsG* element inserts in an intron. Additionally, naturally occurring splice donor sites in the 3' end of the *Ds* element (Wessler et al., 1987; Nussaume et al., 1995) allow splicing and expression if insertion occurs in an exon. In reality, only two of the three SA sites appear to be used (Nussaume et al., 1995), but reporter gene expression in transposants occurs with reasonably high efficiency (Sundaresan et al., 1995). Each *Ds* element was subcloned into a binary T-DNA vector for introduction into Arabidopsis by Agrobacterium-mediated transformation. The counterselectable *iaaH* gene, driven by the ubiquitously expressed 2' promoter, was included on each T-DNA, thereby allowing both the *Ac* and the donor *Ds* loci to be selected against after mobilization.

Transposition is initiated by crossing parental plants homozygous for *Ac* with plants homozygous for one of the *Ds* elements. F_1 plants are allowed to self, and F_2 seeds are collected. F_2 seeds are germinated on media containing NAM and kanamycin. Plants that are resistant to both NAM and kanamycin contain a *Ds* element somewhere in the genome, but that has segregated away from the *Ac* element and the donor *Ds* T-DNA. This selection enriches for transposition events that are not linked to the original *Ds* location. The selection is quite robust, although the need for selection to be done in media rather than soil makes the process labor intensive. Double-resistant F_2 seedlings are transferred to soil and allowed to self-pollinate, and F_3 seeds are collected. Screens for *GUS* expression are performed on F_3 plants.

In a screen of 2000 transposants, 32% of *DsG* insertions and 45% of *DsE* insertions were found to exhibit *GUS* activity in seedlings (Martienssen, 1998). A variety of different expression patterns was identified, including those showing organ, tissue, or cell type specificity. A few representative expression patterns are shown in Figure 3. Surprisingly few transposants showed ubiquitous expression in all cell types (P.S. Springer, Q. Gu, D. Bush, C. Yordan, and R. Martienssen, unpublished results). This observation may indicate that even so-called housekeeping functions required in all plant

cells are likely supplied by gene family members with distinct domains of expression.

Gene Traps Provide Tools for Plant Development

Gene Identification

Perhaps the most exciting use of gene traps is in the identification of genes with specific patterns of expression that are differentially regulated. Reporter gene expression that is cell or tissue specific, or temporally or conditionally regulated, may be identified. As described above, gene traps allow the identification of genes that are not amenable to classical genetic analysis. Therefore, novel genes are likely to be found in any gene trap screen. Screens have in fact been successful in identifying genes specifically expressed in lateral roots (Malamy and Benfey, 1997), developing embryos (Topping et al., 1994; Topping and Lindsey, 1997), and shoot apices (Springer et al., 1995; P.S. Springer and R. Martienssen, unpublished results). Conditional screens have also been performed to identify genes regulated by nematode infections (Barthels et al., 1997; Favery et al., 1998) and

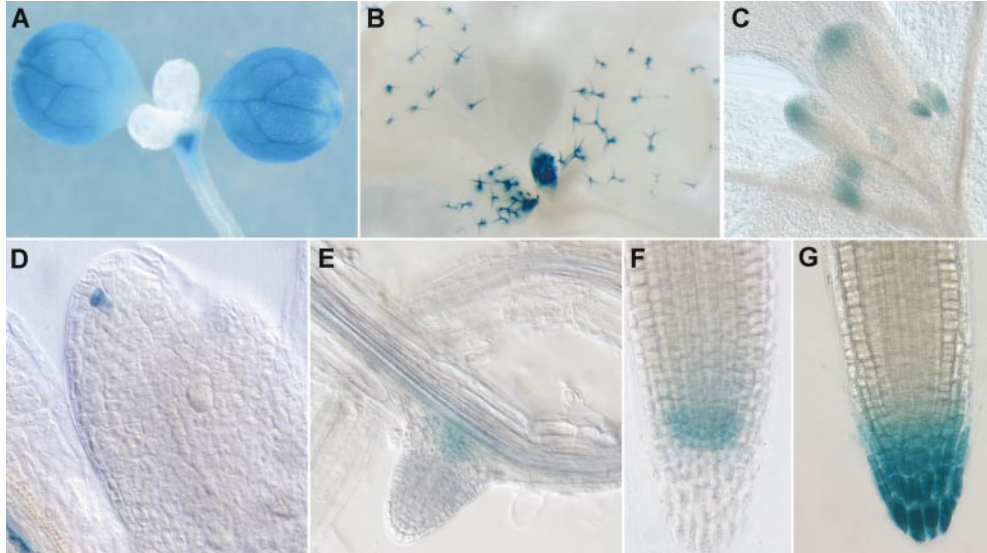


Figure 3. *GUS* Reporter Gene Expression Patterns in Representative Enhancer and Gene Trap Lines.

Expression is evident, as follows:

- (A) In cotyledons and shoot apex but not leaves.
- (B) In trichomes.
- (C) In stipules and leaf tips.
- (D) In a single cell at the tip of leaf primordium.
- (E) In a lateral root primordium.
- (F) In a root tip.
- (G) In a root cap.

in response to abiotic stress (M. Rojas-Pierce, E. Bray, and P.S. Springer, unpublished results). To date, only a few plant genes have been cloned and characterized using gene traps. However, these cases provide examples of identification of both essential and redundant genes. Two specific examples are described below.

The *PROLIFERA* (*PRL*) gene was identified as a gene trap *DsG* insertion that showed GUS activity in dividing cells. *PRL* encodes a protein that is related to MCM7 (Springer et al., 1995), a member of the MCM gene family found in all eukaryotes and required for the initiation of DNA replication (reviewed in Kearsley and Labib, 1998). Expression in dividing cells is consistent with this predicted role in cell division. Disruption of *PRL* by the *DsG* element led to megagametophyte and embryo lethality. Arrest of both megagametophytes and embryos occurred at variable stages of development (Springer et al., 1995, 2000). There are many embryo-lethal mutations in Arabidopsis that have variable phenotypes, which makes it difficult to determine the cause of lethality (Meinke, 1991). However, the GUS expression pattern in dividing cells suggested a role in cell division before the gene was cloned.

An enhancer trap *DsE* insertion was identified in the *AGL8* gene (Gu et al., 1998), which had previously been described as a member of a large family of MADS box genes related to *AGAMOUS* (Mandel and Yanofsky, 1995). The *DsE* insertion in the 5' untranslated leader of *AGL8* caused a loss-of-function mutation that resulted in a failure of the silique to elongate after fertilization. This failure caused the developing silique to be shortened and crowded with seeds. *AGL8* was therefore renamed *FRUITFULL* (*FUL*) to reflect this phenotype (Gu et al., 1998). The defect in silique elongation in *ful* mutants was consistent with its expression pattern in the carpel valves. However, *FUL* was also expressed in the shoot apical meristem and upregulated in meristems undergoing a transition to flowering, suggesting a possible role in the reproductive transition. Indeed, *ful* mutants showed a slight delay in flowering time (Ferrándiz et al., 2000). However, when combined with mutations in related MADS box genes *APETALA1* (*AP1*) and *CAULIFLOWER* (*CAL*), the *ful* mutation causes a complete failure to flower (Ferrándiz et al., 2000). Thus, *FUL* appears to act redundantly with *AP1* and *CAL* to promote the transition to flowering (Ferrándiz et al., 2000). This function was suggested by the expression pattern and was only uncovered by analysis of the triple mutant.

Identification of Markers

An equally important use of gene traps is to identify tissue- or cell-specific expression patterns. Such expression patterns can then be used as markers to identify particular cells or tissues. Markers are useful tools for developmental analysis, although relatively few have been described in plants. Markers that are expressed in distinct patterns during development can be used to examine normal patterns of development and to

characterize mutant phenotypes. Specifically, markers are useful for determining when changes in cell fate occur during morphogenesis and for following cell lineages during development.

Molecular markers are particularly effective tools for analysis of mutant phenotypes. Because developmental mutations often disrupt cellular identity, it can be difficult to determine which specific defects are caused by a gene disruption. The availability of a collection of different cell type-specific markers is very useful for determining cellular identity in mutant tissues. Markers are also useful for identifying very early alterations in developmental pattern. It is sometimes possible to trace a mutant phenotype to a defect that occurs at an early stage in development, perhaps before deviation from normal morphology or anatomy can be detected. Gene traps in plants have been used quite extensively in this capacity. Gene trap patterns have been used to study epidermal patterning (Berger et al., 1998a, 1998b), lateral root initiation (Smith and Fedoroff, 1995; Malamy and Benfey, 1997), leaf development (Tsukaya and Uchimiya, 1997), flower development (Roe et al., 1997; Liljegren et al., 2000), and embryogenesis (Topping et al., 1994; Topping and Lindsey, 1997; Willemsen et al., 1998). Therefore, even in the absence of prior molecular information, gene trap expression patterns can be enormously useful.

Promoter Identification and Ectopically Expressed Genes

In addition to gene identification, gene traps allow for the identification of promoters that drive specific expression. Once identified, specific promoters can be used to drive the ectopic expression of experimental genes to examine patterns of development. An example of this was demonstrated recently by Tsugeki and Fedoroff (1999). The authors isolated a root cap-specific promoter in an enhancer trap line. This promoter was then used to drive expression of a diphtheria toxin gene (*DT-A*) to examine the developmental consequences of ablating root cap cells.

An elegant modification of this approach makes use of the yeast transcriptional activator *GAL4* as a reporter gene. The *GAL4* system was first used in *Drosophila* (Brand and Perrimon, 1993) and has been adapted by Jim Haseloff for use in Arabidopsis (<http://www.plantsci.cam.ac.uk/Haseloff/IndexGAL4.html>). In this system, a T-DNA containing a modified yeast *GAL4* gene (*GAL4-VP16*) fused to the minimal CaMV 35S promoter and a modified *GFP* (*mGFP*) gene driven by the *GAL4* upstream activating sequence (UAS) are transformed into Arabidopsis (Figure 4). When *GAL4-VP16* is positioned under control of a chromosomal enhancer, expression can be visualized by GFP fluorescence, because *GAL4* activates *GFP* expression from the UAS. A battery of enhancer trap lines expressing this *GAL4-VP16* fusion in different patterns has been generated. This system can then be used to express a gene of interest ectopically in many different patterns upon fusion to UAS elements. Genetic

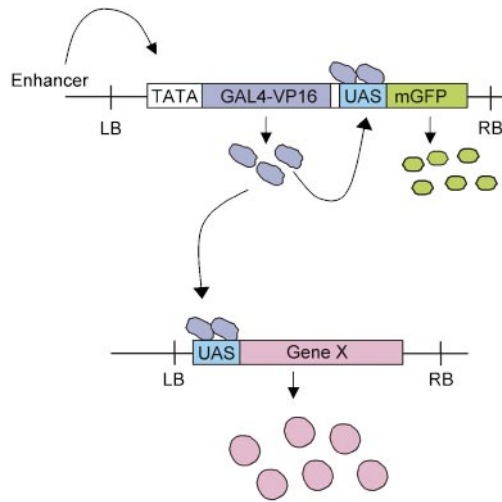


Figure 4. Gene Misexpression Using the *GAL4* System.

A modified *GAL4* gene (*GAL4-VP16*) fused to a minimal promoter that responds to chromosomal enhancer elements is introduced into transgenic plants on a T-DNA vector. The T-DNA also contains an *mGFP* gene fused to UAS sequences. GFP reports *GAL4-VP16* expression, because GAL4 controls transcription of *GFP* through the UAS element. Misexpression of Gene X is achieved by crossing plants containing a UAS::Gene X fusion to a plant with an individual *GAL4* enhancer trap insertion. GAL4 activates Gene X, causing it to be expressed in the same pattern as the GFP reporter. LB, left border of T-DNA; RB, right border of T-DNA.

crosses are used to bring the ectopic gene under control of the *GAL4-VP16* activator, expressed in a particular pattern that is visualized by GFP. This system can also be used to genetically ablate specific cells or morphological regions by expressing a toxin gene in the same way.

Identification of Targets of Regulatory Genes

A potentially powerful use of gene traps is for the identification of downstream genes in a regulatory cascade. This approach has been used very effectively in *Drosophila* to identify target genes that are regulated by the *Antennapedia* gene (Wagner-Bernholz et al., 1991). The rationale is to identify candidate gene trap lines in which the reporter gene is expressed in an appropriate pattern that overlaps the expression domains of the regulatory gene of interest. Gene trap lines can be crossed to mutants that have lost regulatory gene function or to transgenic plants that ectopically express the regulatory gene. Expression patterns of the reporter that are altered in mutant and transgenic backgrounds can indicate genes that are potentially controlled by the regulatory gene. We have used this approach to identify potential downstream targets of the *KNAT1* homeobox

gene (P.S. Springer, B. Shuai, G. Chuck, S. Hake, and R. Martienssen, unpublished data).

Genomics Resources

A variety of gene trap lines exist that contain single *Ds* element insertions in the genome (Sundaresan et al., 1995; Martienssen, 1998; Parinov et al., 1999). To identify the gene that controls reporter gene expression, one can use various polymerase chain reaction (PCR) techniques to isolate genomic DNA flanking the insertions (Martienssen, 1998). Thermal asymmetric interlaced PCR is particularly effective for amplifying flanking DNA sequences (Liu et al., 1995; Tsugeki et al., 1996). Once a small region of flanking sequence is known, then database searches can be performed to identify corresponding genomic DNA sequences and candidate genes. Because the Arabidopsis genome will soon be completely sequenced (Meinke et al., 1998), there is a high probability that even a small region of flanking sequence is sufficient to identify a corresponding genomic sequence. Such identification immediately yields the map position of the corresponding *Ds* insertion. Furthermore, because the genome sequence is being systematically annotated, predicted genes within the region of the insertion can also be identified, which allows for the identification of candidate genes driving reporter gene expression. In the case of gene trap insertions (which result in transcriptional fusions), 5' RACE (for random amplification of cDNA ends)-PCR can also be used to amplify exon sequence upstream of the insertion (Skarnes et al., 1992; Springer et al., 1995). This approach works well for genes that are relatively abundantly expressed. Because RACE-PCR yields exon sequences, candidate genes are directly identified, and cDNA libraries can be screened directly with PCR product probes.

A number of groups are systematically amplifying and sequencing genomic DNA flanking random gene trap insertions in Arabidopsis. Databases containing this sequence information have been generated (Martienssen, 1998; Parinov et al., 1999). The eventual inclusion of expression data and phenotypic information will be an important component of these databases. This sequence information can be used for reverse genetic screens to identify insertions in previously cloned genes and members of gene families. Together, the sequence, expression, and phenotypic data will contribute to current efforts to determine the function of all genes in the Arabidopsis genome.

Do Reporter Gene Patterns Accurately Reflect Chromosomal Gene Expression?

An important consideration is whether reporter gene expression patterns accurately mimic expression of endogenous genes. This question is often asked about gene traps. In *Drosophila* and mouse, a vast majority of enhancer and

gene trap patterns accurately reflect endogenous gene expression (reviewed in Bellen, 1999). There is no reason a priori to believe that the situation is different in plants. However, whereas gene traps have been extensively used as markers for developmental analysis in plants, there are relatively few published descriptions of isolation and characterization of genes controlling reporter gene expression. In most of the cases that have been reported, reporter gene expression does indeed accurately reflect the overall pattern of plant gene expression, although not necessarily the transcript abundance (Smith and Fedoroff, 1995; Di Laurenzio et al., 1996; Gu et al., 1998; Springer et al., 2000; Vielle-Calzada et al., 2000). In fact, gene traps are much more likely to accurately reflect expression than promoter-reporter gene fusions that are extensively used for expression analysis, because all regulatory elements should be in place in a gene trap insertion (with the exception of those that are disrupted by the insertion). In contrast, promoter-reporter gene fusions may not include regulatory elements such as those that are in introns or 3' to the transcribed region. In addition, position effects that can cause transgenes to be incorrectly expressed are not a problem with gene traps, because the controlling elements remain in their natural context in the chromosome. The ability of promoter-reporter gene fusions to accurately report gene expression has recently been questioned, and the use of promoter-reporter gene fusions as the sole determinant of gene expression has been deemed insufficient (Taylor, 1997). In contrast, gene traps are quite likely to accurately reflect endogenous gene expression.

Nonetheless, examples in which expression of a reporter gene and the chromosomal gene do not correlate have been reported. In one example, an enhancer trap insertion that was expressed in a root cap-specific pattern was identified (Tsugeki and Fedoroff, 1999). When the flanking gene was cloned, however, it showed a different expression pattern, leading the authors to conclude that the insertion had disrupted a promoter element needed for proper expression of the *GUS* reporter. In another example, a T-DNA insertion into the tobacco genome resulted in expression of the *GUS* reporter in a seed coat-specific pattern (Fobert et al., 1994). No transcribed gene could be found in the region of the insertion, and the region was not conserved in related species, leading the authors to conclude that the insertion had activated a cryptic promoter. However, it is possible that this particular promoter drives expression of a noncoding RNA or a small peptide that might not have been detected by the methods used. We have observed a similar situation with a *DsG* insertion in one of our transposant lines (P.S. Springer and R. Martienssen, unpublished results).

CONCLUDING REMARKS

Gene traps have proven to be highly useful tools in plant developmental biology. Their largest contribution has been in

the generation of tissue- and cell type-specific markers. Gene identification has been slower in coming, and in some cases, the genes regulating reporter gene expression have been surprisingly difficult to identify. Nonetheless, as the sequence of the Arabidopsis genome is completed, gene traps are likely to play an increasingly important role in the next phase of Arabidopsis genomics—determining gene function.

ACKNOWLEDGMENTS

I thank R. Martienssen for many useful discussions; J. Haseloff, E. Bray, and B. Aronson for helpful comments on the manuscript; D. Holding and M. Geisler for contributions to Figure 3; and J. Haseloff for contributing Figure 4. Research on gene traps in the author's laboratory is supported by National Science Foundation Grant Nos. IBN-9712927 and IBN-9875371 and a grant from the Southwest Consortium on Plant Genetics and Water Resources.

REFERENCES

- Aarts, M.G.M., Corzaan, P., Stiekema, W.J., and Pereira, A. (1995). A two-element *Enhancer-Inhibitor* transposon system in *Arabidopsis thaliana*. *Mol. Gen. Genet.* **247**, 555–564.
- Aida, M., Ishida, T., Fukaki, H., Fujisawa, H., and Tasaka, M. (1997). Genes involved in organ separation in Arabidopsis: An analysis of the *cup-shaped cotyledon* mutant. *Plant Cell* **9**, 841–857.
- Allen, N.D., Cran, D.G., Barton, S.C., Hettler, S., Reik, W., and Surani, M.A. (1988). Transgenes as probes for active chromosomal domains in mouse development. *Nature* **333**, 852–855.
- André, D., Colau, D., Schell, J., Van Montagu, M., and Hernalsteens, J.-P. (1986). Gene tagging in plants by a T-DNA insertion mutagen that generates APH(3')II-plant gene fusions. *Mol. Gen. Genet.* **204**, 512–518.
- Azpiroz-Leehan, R., and Feldmann, K.A. (1997). T-DNA insertion mutagenesis in Arabidopsis: Going back and forth. *Trends Genet.* **13**, 152–156.
- Bancroft, I., and Dean, C. (1993). Transposition pattern of the maize element *Ds* in *Arabidopsis thaliana*. *Genetics* **134**, 1221–1229.
- Bancroft, I., Bhatt, A.M., Sjodin, C., Scofield, S., Jones, J.D.G., and Dean, C. (1992). Development of an efficient two-element transposon tagging system in *Arabidopsis thaliana*. *Mol. Gen. Genet.* **233**, 449–461.
- Barthels, N., et al. (1997). Regulatory sequences of Arabidopsis drive reporter gene expression in nematode feeding structures. *Plant Cell* **9**, 2119–2134.
- Bechtold, N., Ellis, J., and Pelletier, G. (1993). *In planta Agrobacterium* mediated gene transfer by infiltration of adult *Arabidopsis thaliana* plants. *C. R. Acad. Sci. Ser. III Sci. Vie* **316**, 1194–1199.
- Bellen, H.J. (1999). Ten years of enhancer detection: Lessons from the fly. *Plant Cell* **11**, 2271–2281.
- Bellen, H.J., O'Kane, C.J., Wilson, C., Grossniklaus, U., Pearson, R.K., and Gehring, W.J. (1989). P-element-mediated enhancer

- detection: A versatile method to study development in *Drosophila*. *Genes Dev.* **3**, 1288–1300.
- Benfey, P.N., Ren, L., and Chua, N.H.** (1989). The CaMV 35S enhancer contains at least two domains which can confer different developmental and tissue-specific expression patterns. *EMBO J.* **8**, 2195–2202.
- Berger, F., Haseloff, J., Schiefelbein, J., and Dolan, L.** (1998a). Positional information in root epidermis is defined during embryogenesis and acts in domains with strict boundaries. *Curr. Biol.* **8**, 421–430.
- Berger, F., Linstead, P., Dolan, L., and Haseloff, J.** (1998b). Stomata patterning on the hypocotyl of *Arabidopsis thaliana* is controlled by genes involved in the control of root epidermis patterning. *Dev. Biol.* **194**, 226–234.
- Bier, E., Vaessin, H., Shepherd, S., Lee, K., McCall, K., Barbel, S., Ackerman, L., Carretto, R., Uemura, T., Grell, E., Jan, L.Y., and Jan, Y.N.** (1989). Searching for pattern and mutation in the *Drosophila* genome with a P-*lacZ* vector. *Genes Dev.* **3**, 1273–1287.
- Bouchez, D., Camilleri, C., and Caboche, M.** (1993). A binary vector based on Basta resistance for *in planta* transformation of *Arabidopsis thaliana*. *C. R. Acad. Sci. Ser. III Sci. Vie* **316**, 1188–1193.
- Brand, A.H., and Perrimon, N.** (1993). Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* **118**, 401–415.
- Brennan, J., and Skarnes, W.C.** (1999). Gene trapping in mouse embryonic stem cells. *Methods Mol. Biol.* **97**, 123–138.
- Campisi, L., Yang, Y., Yi, Y., Heilig, E., Herman, B., Cassista, A.J., Allen, D.W., Xiang, H., and Jack, T.** (1999). Generation of enhancer trap lines in *Arabidopsis* and characterization of expression patterns in the inflorescence. *Plant J.* **17**, 699–707.
- Carroll, B.J., Klimyuk, V.I., Thomas, C.M., Bishop, G.J., Harrison, K., Scofield, S.R., and Jones, J.D.G.** (1995). Germinal transpositions of the maize element *Dissociation* from T-DNA loci in tomato. *Genetics* **139**, 407–420.
- Casadaban, M.J., and Cohen, S.N.** (1979). Lactose genes fused to exogenous promoters in one step using a Mu-*lac* bacteriophage: *In vivo* probe for transcriptional control sequences. *Proc. Natl. Acad. Sci. USA* **76**, 4530–4533.
- Das, L., and Martienssen, R.A.** (1995). Site-selected transposon mutagenesis at the *hcf106* locus in maize. *Plant Cell* **7**, 287–294.
- DeBlock, M., Botterman, J., Vandewiele, M., Dockx, J., Thoen, C., Gossele, V., RaoMouva, N., Thompson, C., Van Montagu, M., and Leemans, J.** (1987). Engineering herbicide resistance in plants by expression of a detoxifying enzyme. *EMBO J.* **6**, 2513–2518.
- Di Laurenzio, L., Wysocka-Diller, J., Malamy, J.E., Pysh, L., Helariutta, Y., Freshour, G., Hahn, M.G., Feldmann, K.A., and Benfey, P.N.** (1996). The *SCARECROW* gene regulates an asymmetric cell division that is essential for generating the radial organization of the Arabidopsis root. *Cell* **86**, 423–433.
- Dooner, H.K., and Belachew, A.** (1989). Transposition of the maize element *Ac* from the *bz-m2* (*Ac*) allele. *Genetics* **122**, 447–457.
- Dooner, H.K., Keller, J., Harper, E., and Ralston, E.** (1991). Variable patterns of transposition of the maize element *Activator* in tobacco. *Plant Cell* **3**, 473–482.
- Favery, B., Lecomte, P., Gil, N., Bechtold, N., Bouchez, D., Dalmasso, A., and Abad, P.** (1998). RPE, a plant gene involved in early developmental steps of nematode feeding cells. *EMBO J.* **17**, 6799–6811.
- Fedoroff, N.V., and Smith, D.L.** (1993). A versatile system for detecting transposition in *Arabidopsis*. *Plant J.* **3**, 273–289.
- Feldmann, K.A., and Marks, M.D.** (1987). *Agrobacterium*-mediated transformation of germinating seeds of *Arabidopsis thaliana*: A non-tissue culture approach. *Mol. Gen. Genet.* **208**, 1–9.
- Ferrándiz, C., Gu, Q., Martienssen, R., and Yanofsky, M.F.** (2000). Redundant regulation of meristem identity and plant architecture by *FRUITFULL*, *APETALA1* and *CAULIFLOWER*. *Development* **127**, 725–734.
- Fobert, P.R., Miki, B.L., and Iyer, V.N.** (1991). Detection of gene regulatory signals in plants revealed by T-DNA-mediated fusions. *Plant Mol. Biol.* **17**, 837–851.
- Fobert, P.R., Labbé, H., Cosmopoulos, J., Gottlob-McHugh, S., Ouellet, T., Hattori, J., Sunohara, G., Iyer, V.N., and Miki, B.L.** (1994). T-DNA tagging of a seed coat-specific cryptic promoter in tobacco. *Plant J.* **6**, 567–577.
- Goldsbrough, A.P., Tong, Y., and Yoder, J.I.** (1996). *Lc* as a non-destructive visual reporter and transposition excision marker gene for tomato. *Plant J.* **9**, 927–933.
- Gossler, A., Joyner, A.L., Rossant, J., and Skarnes, W.C.** (1989). Mouse embryonic stem cells and reporter constructs to detect developmentally regulated genes. *Science* **244**, 463–465.
- Greenblatt, I.M.** (1984). A chromosome replication pattern deduced from pericarp phenotypes resulting from movements of the transposable element *Modulator* in maize. *Genetics* **108**, 471–485.
- Grossniklaus, U., Bellen, H.J., Wilson, C., and Gehring, W.J.** (1989). P-element-mediated enhancer detection applied to the study of oogenesis in *Drosophila*. *Development* **107**, 189–200.
- Gu, Q., Ferrándiz, C., Yanofsky, M.F., and Martienssen, R.** (1998). The *FRUITFULL* MADS-box gene mediates cell differentiation during *Arabidopsis* fruit development. *Development* **125**, 1509–1517.
- Haseloff, J., and Amos, B.** (1995). GFP in plants. *Trends Genet.* **11**, 328–329.
- Haseloff, J., Siemerling, K.R., Prasher, D.C., and Hodge, S.** (1997). Removal of a cryptic intron and subcellular localization of green fluorescent protein are required to mark transgenic *Arabidopsis* plants brightly. *Proc. Natl. Acad. Sci. USA* **94**, 2122–2127.
- Herman, L., Jacobs, A., Van Montagu, M., and Depicker, A.** (1990). Plant chromosome/marker gene fusion assay for study of normal and truncated T-DNA integration events. *Mol. Gen. Genet.* **224**, 248–256.
- Jefferson, R.A., Kavanagh, T.A., and Bevan, M.W.** (1987). GUS fusions: β -Glucuronidase as a sensitive and versatile gene fusion marker in higher plants. *EMBO J.* **6**, 3901–3907.
- Jones, D.A., Thomas, C.M., Hammond-Kosack, K.E., Balint-Kurti, P.J., and Jones, J.D.G.** (1994). Isolation of the tomato *Cf-9* gene for resistance to *Cladosporium fulvum* by transposon tagging. *Science* **266**, 789–793.
- Jones, J.D.G., Carland, F.M., Maliga, P., and Dooner, H.K.** (1989). Visual detection of transposition of the maize element *Activator* (*Ac*) in tobacco seedlings. *Science* **244**, 204–207.

- Jones, J.D.G., Carland, F., Lim, E., Ralston, E., and Dooner, H.K. (1990). Preferential transposition of the maize element *Activator* to linked chromosomal locations in tobacco. *Plant Cell* **2**, 701–707.
- Kearsey, S.E., and Labib, K. (1998). MCM proteins: Evolution, properties, and role in DNA replication. *Biochim. Biophys. Acta* **1398**, 113–136.
- Kertbundit, S., De Greve, H., Deboeck, F., Van Montagu, M., and Hernalsteens, J.-P. (1991). *In-vivo* random β -glucuronidase gene fusions in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. USA* **88**, 5212–5216.
- Klee, H.J., Horsch, R.B., Hinchee, M.A., Hein, M.B., and Hoffmann, N.L. (1987). The effects of overproduction of two *Agrobacterium tumefaciens* T-DNA auxin biosynthetic gene products in transgenic petunia plants. *Genes Dev.* **1**, 86–96.
- Klimyuk, V.I., Nussaume, L., Harrison, K., and Jones, J.D.G. (1995). Novel GUS expression patterns following transposition of an enhancer trap *Ds* element in *Arabidopsis*. *Mol. Gen. Genet.* **249**, 357–365.
- Koncz, C., Martini, N., Mayerhofer, R., Koncz-Kalman, Z., Körber, H., Redei, G.P., and Schell, J. (1989). High-frequency T-DNA-mediated gene tagging in plants. *Proc. Natl. Acad. Sci. USA* **86**, 8467–8471.
- Kothary, R., Clapoff, S., Brown, A., Campbell, R., Peterson, A., and Rossant, J. (1988). A transgene containing *lacZ* inserted into the *dystonia* locus is expressed in neural tube. *Nature* **335**, 435–437.
- Krysan, P.J., Young, J.C., and Sussman, M.R. (1999). T-DNA as an insertional mutagen in *Arabidopsis*. *Plant Cell* **11**, 2283–2290.
- Laufs, P., Autran, D., and Traas, J. (1999). A chromosomal paracentric inversion associated with T-DNA integration in *Arabidopsis*. *Plant J.* **18**, 131–139.
- Liljegren, S.J., Ditta, G.S., Eshed, Y., Savidge, B., Bowman, J.L., and Yanofsky, M.F. (2000). *SHATTERPROOF* MADS-box genes control seed dispersal in *Arabidopsis*. *Nature* **404**, 766–770.
- Lin, X., et al. (1999). Sequence and analysis of chromosome 2 of the plant *Arabidopsis thaliana*. *Nature* **402**, 761–768.
- Lindsey, K., Wei, W., Clarke, M.C., McArdle, H.F., Rooke, L.M., and Topping, J.F. (1993). Tagging genomic sequences that direct transgene expression by activation of a promoter trap in plants. *Transgenic Res.* **2**, 33–47.
- Liu, Y.-G., Mitsukawa, N., Oosumi, T., and Whittier, R.F. (1995). Efficient isolation and mapping of *Arabidopsis thaliana* T-DNA insert junctions by thermal asymmetric interlaced PCR. *Plant J.* **8**, 457–463.
- Lloyd, A.M., Walbot, V., and Davis, R.W. (1992). *Arabidopsis* and *Nicotiana* anthocyanin production activated by maize regulators *R* and *C1*. *Science* **258**, 1773–1775.
- Long, D., Swinburne, J., Martin, M., Wilson, K., Sundberg, E., Lee, K., and Coupland, G. (1993). Analysis of the frequency of inheritance of transposed *Ds* elements in *Arabidopsis* after activation by a CaMV 35S promoter fusion to the *Ac* transposase gene. *Mol. Gen. Genet.* **241**, 627–636.
- Long, D., Goodrich, J., Wilson, K., Sundberg, E., Martin, M., Puangsomlee, P., and Coupland, G. (1997). *Ds* elements on all five *Arabidopsis* chromosomes and assessment of their utility for transposon tagging. *Plant J.* **11**, 145–148.
- Machida, C., Onouchi, H., Koizumi, J., Hamada, S., Semiarti, E., Torikai, S., and Machida, Y. (1997). Characterization of the transposition pattern of the *Ac* element in *Arabidopsis thaliana* using endonuclease I-SceI. *Proc. Natl. Acad. Sci. USA* **94**, 8675–8680.
- Malamy, J.E., and Benfey, P.N. (1997). Organization and cell differentiation in lateral roots of *Arabidopsis thaliana*. *Development* **124**, 33–44.
- Mandel, M.A., and Yanofsky, M.F. (1995). The *Arabidopsis AGL8* MADS box gene is expressed in inflorescence meristems and is negatively regulated by *APETALA1*. *Plant Cell* **7**, 1763–1771.
- Martienssen, R.A. (1998). Functional genomics: Probing plant gene function and expression with transposons. *Proc. Natl. Acad. Sci. USA* **95**, 2021–2026.
- Mayer, K., et al. (1999). Sequence and analysis of chromosome 4 of the plant *Arabidopsis thaliana*. *Nature* **402**, 769–777.
- McKinney, E.C., Ali, N., Traut, A., Feldmann, K.A., Belostotsky, D.A., McDowell, J.M., and Meagher, R.B. (1995). Sequence-based identification of T-DNA insertion mutations in *Arabidopsis*: Actin mutants *act2-1* and *act4-1*. *Plant J.* **8**, 613–622.
- Meinke, D.W. (1991). Embryonic mutants in *Arabidopsis thaliana*. *Dev. Genet.* **12**, 382–392.
- Meinke, D.W., Cherry, J.M., Dean, C., Rounsley, S.D., and Koornneef, M. (1998). *Arabidopsis thaliana*: A model plant for genome analysis. *Science* **282**, 662–682.
- Nacry, P., Camilleri, C., Courtial, B., Caboche, M., and Bouchez, D. (1998). Major chromosomal rearrangements induced by T-DNA transformation in *Arabidopsis*. *Genetics* **149**, 641–650.
- Nussaume, L., Harrison, K., Klimyuk, V., Martienssen, R., Sundaresan, V., and Jones, J.D. (1995). Analysis of splice donor and acceptor site function in a transposable gene trap derived from the maize element *Activator*. *Mol. Gen. Genet.* **249**, 91–101.
- Ohba, T., Yoshioka, Y., Machida, C., and Machida, Y. (1995). DNA rearrangement associated with the integration of T-DNA in tobacco: An example for multiple duplications of DNA around the integration target. *Plant J.* **7**, 157–164.
- O’Kane, C.J., and Gehring, W.J. (1987). Detection *in situ* of genomic regulatory elements in *Drosophila*. *Proc. Natl. Acad. Sci. USA* **84**, 9123–9127.
- O’Keefe, D.P., Tepperman, J.M., Dean, C., Leto, K.J., Erbes, D.L., and Odell, J.T. (1994). Plant expression of a bacterial cytochrome P450 that catalyzes activation of a sulfonylurea pro-herbicide. *Plant Physiol.* **105**, 473–482.
- Osborne, B.I., and Baker, B. (1995). Movers and shakers: Maize transposons as tools for analyzing other plant genomes. *Curr. Opin. Cell Biol.* **7**, 406–413.
- Osborne, B.I., Corr, C.A., Prince, J.P., Hehl, R., Tanksley, S.D., McCormick, S., and Baker, B. (1991). *Ac* transposition from a T-DNA can generate linked and unlinked clusters of insertions in the tomato genome. *Genetics* **129**, 833–844.
- Parinov, S., Sevugan, M., Ye, D., Yang, W.-C., Kumaran, M., and Sundaresan, V. (1999). Analysis of flanking sequences from *Dissociation* insertion lines: A database for reverse genetics in *Arabidopsis*. *Plant Cell* **11**, 2263–2270.
- Reuber, T.L., and Ausubel, F.M. (1995). Differential mRNA display. *Methods Cell Biol.* **49**, 431–440.

- Richmond, T., and Somerville, S. (2000). Chasing the dream: Plant EST microarrays. *Curr. Opin. Plant Biol.* **3**, 108–116.
- Roe, J.L., Nemhauser, J.L., and Zambryski, P.C. (1997). *TOU-SLED* participates in apical tissue formation during gynoecium development in *Arabidopsis*. *Plant Cell* **9**, 335–353.
- Rossant, J., and Hopkins, N. (1992). Of fin and fur—Mutational analysis of vertebrate embryonic development. *Genes Dev.* **6**, 1–13.
- Ross-MacDonald, P., et al. (1999). Large-scale analysis of the yeast genome by transposon tagging and gene disruption. *Nature* **402**, 413–418.
- Siemering, K.R., Golbik, R., Sever, R., and Haseloff, J. (1996). Mutations that suppress the thermosensitivity of green fluorescent protein. *Curr. Biol.* **6**, 1653–1663.
- Skarnes, W.C. (1990). Entrapment vectors: A new tool for mammalian genetics. *Bio/Technology* **8**, 827–831.
- Skarnes, W.C. (1993). The identification of new genes: Gene trapping in transgenic mice. *Curr. Opin. Biotechnol.* **4**, 684–689.
- Skarnes, W.C., Auerbach, B.A., and Joyner, A.L. (1992). A gene trap approach in mouse embryonic stem cells: The *lacZ* reporter is activated by splicing, reflects endogenous gene expression, and is mutagenic in mice. *Genes Dev.* **6**, 903–918.
- Smith, D.L., and Fedoroff, N.V. (1995). LRP1, a gene expressed in lateral and adventitious root primordia of *Arabidopsis*. *Plant Cell* **7**, 735–745.
- Smith, D.L., Yanai, Y., Liu, Y.-G., Ishiguro, S., Okada, K., Shibata, D., Whittier, R.F., and Fedoroff, N.V. (1996). Characterization and mapping of *Ds*-GUS-T-DNA lines for targeted insertional mutagenesis. *Plant J.* **10**, 721–732.
- Spradling, A.C., Stern, D.M., Kiss, I., Roote, J., Lavery, T., and Rubin, G.M. (1995). Gene disruptions using *P* transposable elements: An integral component of the *Drosophila* genome project. *Proc. Natl. Acad. Sci. USA* **92**, 10824–10830.
- Springer, P.S., McCombie, W.R., Sundaresan, V., and Martienssen, R.A. (1995). Gene trap tagging of *PROLIFERA*, an essential *MCM2-3-5*-like gene in *Arabidopsis*. *Science* **268**, 877–880.
- Springer, P.S., Holding, D.R., Groover, A., Yordan, C., and Martienssen, R.A. (2000). The essential *Mcm7* protein *PROLIFERA* is localized to the nucleus of dividing cells during the G_1 phase and is required maternally for early *Arabidopsis* development. *Development* **127**, 1815–1822.
- Stougaard, J. (1993). Substrate-dependent negative selection in plants using a bacterial cytosine deaminase gene. *Plant J.* **3**, 755–761.
- Sundaresan, V., Springer, P., Volpe, T., Haward, S., Jones, J.D.G., Dean, C., Ma, H., and Martienssen, R. (1995). Patterns of gene action in plant development revealed by enhancer trap and gene trap transposable elements. *Genes Dev.* **9**, 1797–1810.
- Taylor, C.B. (1997). Promoter fusion analysis: An insufficient measure of gene expression. *Plant Cell* **9**, 273–275.
- Teeri, T.H., Herrera-Estrella, L., Depicker, A., Van Montagu, M., and Palva, E.T. (1986). Identification of plant promoters *in situ* by T-DNA-mediated transcriptional fusions to the *npt-II* gene. *EMBO J.* **5**, 1755–1760.
- Tissier, A.F., Marillonnet, S., Klimyuk, V., Patel, K., Torres, M.A., Murphy, G., and Jones, J.D. (1999). Multiple independent defective *Suppressor-mutator* transposon insertions in *Arabidopsis*: A tool for functional genomics. *Plant Cell* **11**, 1841–1852.
- Topping, J.F., and Lindsey, K. (1997). Promoter trap markers differentiate structural and positional components of polar development in *Arabidopsis*. *Plant Cell* **9**, 1713–1725.
- Topping, J.F., Wei, W., and Lindsey, K. (1991). Functional tagging of regulatory elements in the plant genome. *Development* **112**, 1009–1019.
- Topping, J.F., Agyeman, F., Henricot, B., and Lindsey, K. (1994). Identification of molecular markers of embryogenesis in *Arabidopsis thaliana* by promoter trapping. *Plant J.* **5**, 895–903.
- Tsugeki, R., and Fedoroff, N.V. (1999). Genetic ablation of root cap cells in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA* **96**, 12941–12946.
- Tsugeki, R., Kochieva, E.Z., and Fedoroff, N.V. (1996). A transposon insertion in the *Arabidopsis SSR16* gene causes an embryo-defective lethal mutation. *Plant J.* **10**, 479–489.
- Tsakaya, H., and Uchimiya, H. (1997). Genetic analyses of the formation of the serrated margin of leaf blades in *Arabidopsis*: Combination of a mutational analysis of leaf morphogenesis with the characterization of a specific marker gene expressed in hydathodes and stipules. *Mol. Gen. Genet.* **256**, 231–238.
- Vielle-Calzada, J.-P., Baskar, R., and Grossniklaus, U. (2000). Delayed activation of the paternal genome during seed development. *Nature* **404**, 91–94.
- Wagner-Bernholz, J.T., Wilson, C., Gibson, G., Schuh, R., and Gehring, W.J. (1991). Identification of target genes of the homeotic gene *Antennapedia* by enhancer detection. *Genes Dev.* **5**, 2467–2480.
- Weigel, D., et al. (2000). Activation tagging in *Arabidopsis*. *Plant Physiol.* **122**, 1003–1014.
- Wessler, S.R., Baran, G., and Varagona, M. (1987). The maize transposable element *Ds* is spliced from RNA. *Science* **237**, 916–918.
- Wilhelmi, L.K., and Preuss, D. (1996). Self-sterility in *Arabidopsis* due to defective pollen tube guidance. *Science* **274**, 1535–1537.
- Willemsen, V., Wolkenfelt, H., de Vrieze, G., Weisbeek, P., and Scheres, B. (1998). The *HOBBIT* gene is required for formation of the root meristem in the *Arabidopsis* embryo. *Development* **125**, 521–531.
- Wilson, C., Pearson, R.K., Bellen, H.J., O’Kane, C.J., Grossniklaus, U., and Gehring, W.J. (1989). P-element-mediated enhancer detection: An efficient method for isolating and characterizing developmentally regulated genes in *Drosophila*. *Genes Dev.* **3**, 1301–1313.
- Winkler, R.G., Frank, M.R., Galbraith, D.W., Feyereisen, R., and Feldmann, K.A. (1998). Systematic reverse genetics of transfer-DNA-tagged lines of *Arabidopsis*: Isolation of mutations in the cytochrome P450 gene superfamily. *Plant Physiol.* **118**, 743–750.