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More efficient transposon mutagenesis in *Arabidopsis*?

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Leonore Reiser

Abstract

A maize transposable element has been used to generate a tool for identifying gene function in *Arabidopsis thaliana*.

Significance and context

Mutational analysis is crucial for defining the biological role of a gene. In the case of a gene that has been sequenced, but for which no mutant is known, mutations can be found using 'reverse-genetic' techniques and the phenotype determined. In *Arabidopsis thaliana*, this mainly involves transformation with the bacterium *Agrobacterium tumefaciens*, which integrates its transfer DNA (T-DNA) into the plant genome. Individual mutagenized plants can then be screened for the desired mutation by PCR using primers specific for the gene of interest and the inserted DNA. Speulman *et al.* report a new reverse-genetic resource in the form of transgenic *Arabidopsis* containing the maize transposable elements *Enhancer* (*En*, also called *Spm*) and *Inhibitor* (*I*, also called *dSpm*). The autonomous *En* element encodes a transposase that induces its own transposition and excision as well as that of the non-autonomous *I* element. Unlike T-DNA insertions, the *En-I* system increases in the number of copies of *I*, and therefore the number of insertions, in subsequent generations; thus, fewer plants should need to be produced to obtain mutational saturation of the genome.

Key results

To assess the frequency and distribution of transpositions, the regions flanking the insertions in 17 lines, which had been outcrossed to segregate away the *En* element, were cloned, and the sequences compared with known *Arabidopsis* sequences. Of these sequences, 74% showed similarity to known sequences and 65% could be located to a chromosomal region. Insertions were found all over the genome, but about 10% were positioned near the site of the original insertion in the progenitor plant, suggesting that *I* transposes to linked sites. The methodology was tested by screening for insertions both in cloned genes with well described mutant phenotypes and in a cloned gene for which no mutant phenotype was known. For example, insertions into the gene *FATTY ACID ELONGATION1* (*FAE1*) were used to show that a phenotype identical to that of the *FIDDLEHEAD (fdh)* mutant wasassociated with this gene.Independent experiments have recently confirmed thatthe *fdh* locus is encoded by the

FAE1 gene. It is also possible to use these stocks in a 'forward', or traditional, genetic approach by visual inspection for a phenotype of interest.

Methodological innovations

Arabidopsis plants were transformed with a single construct containing both the *En* and *I* elements and a selectable marker.A single line containing two insertions (T-*En2* and T-*En5*), located on chromosomes 1 and 2, respectively, was self-pollinated repeatedly to increase the copy number of the transposable element. The resulting 216 T-*En5* homozygotes identified were selected as progenitors of 2,592 single-seed descent lines produced by self-pollination. DNAs from the single-seed lineswere arranged in a three-dimensional array to facilitate location of a specific insertion by a coordinate system.

Links

The Nottingham *Arabidopsis* stock center (NASC) maintains several collections of T-DNA and transposon insertion lines that are publicly available including subpools of lines described in this paper. The Sequenced insertion sites (SINS) database shows BLAST similarities of the flanking regions of 1,200 *Spm* insertions, seed from which are also available from NASC. A Ds insertion database is also available. The Wisconsin T-DNA knockout facility will perform a primary screen of their library of T-DNA insertions and supply seed for further analysis of positive lines. The *Arabidopsis* Information Resource (TAIR) contains many tools and resources for *Arabidopsis* researchers.

Reporter's comments

The insertional mutagenesis technique described here will be a useful tool for plant researchers who want to attribute function to genes identified by sequence alone. As expected, the number of insertions per plant is higher than that of previously described T-DNA insertion populations. However, it has been shown experimentally that about 50% of the putative positive insertions are not heritable, effectively reducing the number of insertions that result in an analyzable phenotype. This is most likely to be due to instability of the transposable element. Unstable insertions can, however, be useful for identifying revertants. *I* appears to insert into regions of the genome rich in A:T basepairs, which may result in a preference for insertions into regulatory regions and introns rather than into exons. Thus, different classes of mutation might be obtained from this resource. Combined with the output of other reverse-genetics tools, this provides a valuable resource for plant biologists seeking mutations in particular genes.

Table of links

Plant Cell

Nottingham *Arabidopsis* stock center

Sequenced insertion sites

Ds insertion database

Wisconsin T-DNA knockout facility

The *Arabidopsis* Information Resource

References

1. Speulman E, Metz PLJ, Arkel G van, Hekkert B te Lintel, Stiekema WJ, Pereira A: A two component *Enhancer-Inhibitor* transposon mutagenesis system for functional analysis of the *Arabidopsis* genome. Plant Cell. 1999, 11: 1853-1866. 1040-4651