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Mutagenesis of a large *MYB* gene family in *Arabidopsis*

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Abstract

PCR screening five collections of mutagenized *Arabidopsis* lines to find those that have T-DNA or transposon insertions in 73 members of the R2R3 *MYB* gene family has identified 47 insertions into 36 genes.

Significance and context

Approximately 84% and 55% of the genome sequence and genome annotation, respectively, of *Arabidopsis thaliana* is publicly available in GenBank as of January 2000, and this has already fostered a number of genome-wide studies on gene expression and gene function. To facilitate the isolation of null alleles, many groups have created lines carrying T-DNA or transposable elements throughout the *Arabidopsis* genome. Meissner *et al.* survey the practical advantages and disadvantages of five of these 'knock-out' collections in an attempt to find lines that have insertions in the R2R3 subfamily of *MYB* genes, which encode transcription factors. *MYB* genes are involved in the regulation of cell proliferation and differentiation in animals. In plants, they are involved in a number of processes, including the regulation of secondary metabolism, abiotic stress, pathogen defense, and cellular morphogenesis. Finding mutants in this gene family presents a particular challenge because of sequence similarity among the large number of family members, potential redundancy of function, and the relatively small size of the genes (1.6 kilobases, where the expected average size of an *Arabidopsis* gene size is 4.8 kilobases).

Key results

The five 'knock-out' collections screened are: *En*/*Spm* (for Enhancer or Suppressor-mutator) transposon insertional populations from AMAZE lines (Max Planck Inst. Zuchtungsforsch, Cologne, Germany); *En/Spm-I/dSpm* transposon system from SLAT (Sainsbury Laboratory *Arabidopsis thaliana*) and from the Centre for Plant Breeding and Reproduction Research, Wageningen, The Netherlands; and T-DNA insertional lines from the Laboratoire de Biologie Cellulaire, Versailles, France and Consejo Superior de Investigaciones Cientificas (CSIC), Madrid, Spain. The SLAT and T-DNA populations have stable insertions at low copy numbers (1-3 per line), whereas the AMAZE and Wageningen lines have mobile transposons at higher copy numbers (approximately 2-30 per line). The general strategy for identifying lines with insertions in the R2R3 *MYB* genes was to use gene-specific primers and primers

against the insertion elements in a semi-nested or nested PCR. The AMAZE population gave the highest rate of insertion into the genes that were screened (54% insertion per gene screened from 8000 lines); in the other populations, insertions in *MYB* genes screened were detected at a rate of about 20%. The multicopy, mobile transposon lines generally had a higher rate of insertion in the *MYB* genes than the populations with non-mobile insertions, but the presence of other insertions and the mobile nature of the insertion will complicate the further characterization of these genes. On the other hand, SLAT populations (with stable transposons) are better suited for characterization of gene function, but isolating the individual lines carrying the insertion requires much more effort, as each DNA pool tested comes from a population of 50 independent insertional lines. Most of the lines with insertions in a gene of interest had reduced amounts of the gene transcript, but these lines need to be characterized further to identify the consequence of the insertion. None of the lines homozygous for an insertion in the gene of interest gave an obvious visible phenotype under normal growth conditions.

Links

Information on the *Arabidopsis* sequencing project is available from The *Arabidopsis* Information Resource's *Arabidopsis* Genome Initiative. The Versailles T-DNA insertional lines and the SLAT transposon insertional lines are available from the Nottingham *Arabidopsis* stock centre.

Reporter's comments

The advantages and disadvantages of searching this set of insertionally mutagenized populations to find insertions in a gene of interest are clearly presented by the authors. Although no single population has reached saturation for insertional mutagenesis, Meissner *et al.* were able to find insertions in had an approximately 50% of the genes screened, by screening approximately 52,000 lines derived using a variety of mutagenesis methods. It is also clear that finding a homozygous line with an insertion in a gene of interest is a long way from finding a biological role for the gene, as not all these lines lacked the transcript or gave a clear phenotype. Determining the expression pattern of a gene and its protein in wild-type plants, as well as studying the global expression pattern of other genes in a line lacking the relevant protein, and studying lines carrying multiple null alleles of members of the gene family, may help us to understand the roles of the *MYB* family of transcription factors in *Arabidopsis*.

Table of links

Plant Cell

GenBank

Arabidopsis Genome Initiative

Nottingham *Arabidopsis* stock centre

References

1. Meissner RC, Jin H, Cominelli E, Denekamp M, Fuertes A, Greco R, Kranz HD, Penfield S, Petroni K, Urzainqui A, et al: Functional search in a large transcription factor gene family in *Arabidopsis*: Assessing the potential of reverse genetics to identify insertional mutations in R2R3 *MYB* genes. Plant Cell. 1999, 11: 1827-1840. 1040-4651