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Identification of a Mobile Endogenous Transposon in *Arabidopsis thaliana*

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A mobile endogenous transposable element, *Tag1*, has been identified in the plant *Arabidopsis thaliana*. *Tag1* was found in the nitrate transporter gene, *CHL1*, of a chlorate-resistant mutant present in a population of plants containing an active maize *Ac* transposon. *Tag1* excises from the *chl1* gene producing chlorate-sensitive revertants with *Tag1* or *Tag1*-related elements at different loci. *Tag1* and related elements are present in the Landsberg but not Columbia or Wassilewskija ecotypes of *Arabidopsis*. Thus, *Tag1* provides a tool for the insertional mutagenesis of plant genes essential for biological processes of agronomic importance.

Transposable elements have been invaluable for the identification and isolation of genes, as insertion of a transposon both disrupts and tags a gene with a known sequence (*1*). *Arabidopsis thaliana*, with its exceptionally small genome (100,000 kb), would be especially useful for the tagging of plant genes with transposons (*2*). *Arabidopsis* genes have been tagged by transferred DNA (T-DNA) from the soil bacterium *Agrobacterium tumefacians* (*3*) or isolated with the use of a map-based strategy (*4*). Endogenous transposons of *Arabidopsis* include *Tal-10* (*5*) and a transposon-like element *Tat1* (*6*), but these are not mobile; that is, they do not transpose during development or transmission from one generation to the next. The maize *Ac* element, however, is mobile in *Arabidopsis* (*7*).

In an effort to exploit *Ac* as an insertional mutagen, we used several transgenic *Arabidopsis* lines carrying active *Ac* elements to search for mutants defective in the assimilation of nitrate. Such mutants can be selected with the herbicide chlorate. Chlorate is taken up by plants then reduced by nitrate reductase to chlorite, which is toxic (*8*). Mutants that are resistant to chlorate treatment are usually defective in chlorate (and nitrate) reduction (*9*). One exception is the *chl1* mutant of *Arabidopsis*, which is defective in chlorate and nitrate uptake (*10*). When we applied chlorate to the *Ac*-carrying *Arabidopsis* lines, we found a chlorate-resistant mutant with an endogenous transposable element integrated into the *CHL1* gene.

Arabidopsis seed (ecotype Landsberg) used for the chlorate selection originated from three independent transgenic plants containing an *Ac* element cloned into the 5′ untranslated leader region of a streptomycin-resistance gene (*11*). Progeny fully resistant to streptomycin (64 plants) were selected. These 64 plants were the product of an excision event of *Ac*, which restored the functional streptomycin resistance gene. Progeny (20 to 50 seeds from each of the 64 plants) were planted and self-fertilized, seed was harvested, and 100 to 200 seeds from each lineage were then germinated and treated with chlorate. Three chlorate-resistant mutants appeared in one family. A backcross to a *chl1* mutant (*chl1-1*) (*10*) indicated that the mutations were alleles of *chl1*. One of the three mutants, *chl1-6*, was characterized further.

We cloned the *CHL1* gene from a *chl1* mutant tagged with T-DNA (*12*). A *CHL1* cDNA clone (*12*) was used to analyze the

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defect in the *chl1-6* mutant. The mutant *chl1-6* was expected to be homozygous because the mutation is recessive and arose from a lineage that had been self-fertilized for several generations. Southern DNA blot and sequence analysis of *chl1-6* and wild-type DNA with radiolabeled *CHL1* cDNA (*12*) showed that *chl1-6* contained a 3.3-kb insert in the fourth intron of the *CHL1* gene (Fig. 1). Surprisingly, a radiolabeled *Ac* clone did not hybridize to the insert DNA. Sequence analysis of the insert and adjacent DNA revealed that the insert is not *Ac*, but is an element that has the characteristics of a transposon: genomic sequence flanking the insert was duplicated and the element contained inverted terminal repeats (Fig. 2). Comparison of insert and flanking DNA sequence at several different loci revealed that 8 base pairs (bp) of duplicated genomic DNA flanks 22-bp inverted repeats of the element (Fig. 2). The element was named *Tag1* for tagging of *Arabidopsis* genes.

To determine if *Tag1* could excise from the *chl1* gene, progeny from two individual homozygous *chl1-6::Tag1* mutants were examined. Because *Tag1* is located in an intron, excision of the element should restore a functional *CHL1* gene and produce revertant progeny that are sensitive to chlorate. Of the progeny from the two mutants, 28% and 25% were revertant. Five revertants—*chl1-6R1*, *chl1-6R2*, *chl1-6R3*, *chl1-6R4*, and *chl1-6R5*—were picked from a pool of F2 seed and further characterized. These revertants were selected for analysis because they were homozygous and gave rise only to chlorate-sensitive progeny. Southern blot analysis of the revertants showed that *Tag1* had excised from the *chl1*

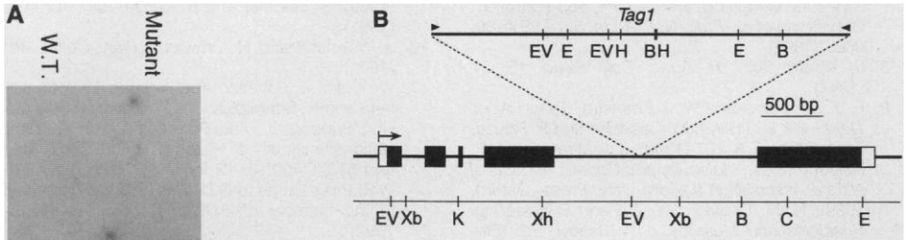

Fig. 1. Genomic analysis of the *Tag1* insertion in the *chl1* gene. (**A**) Southern blot analysis of Sal I–digested *Arabidopsis* genomic DNA isolated from the parent line, ecotype Landsberg *erecta* (wild type) and from 20 progeny of the *chl1-6::Tag1* (Mutant). Blot was hybridized with radiolabeled *CHL1* cDNA (*12*) as described (*16*). (**B**) Schematic representation of the *Tag1* insertion in mutant *chl1-6::Tag1*. DNA used in (A) was digested with Sal I and ligated to the vector λDash (Stratagene, La Jolla, California). A positive clone, λLC-52, was identified by hybridization to radiolabeled *CHL1* cDNA and its sequence determined. Triangles represent the inverted repeat at *Tag1* termini. Filled boxes mark exons of *CHL1*; open boxes represent the 5′ and 3′ untranslated regions. Initiation site and direction of *CHL1* transcription are indicated by the arrow. Restriction sites shown are: B, Bgl II; C, Cla I; E, Eco RI; EV, Eco RV; H, Hind III; K, Kpn I; Xb, Xba I; and Xh, Xho I.

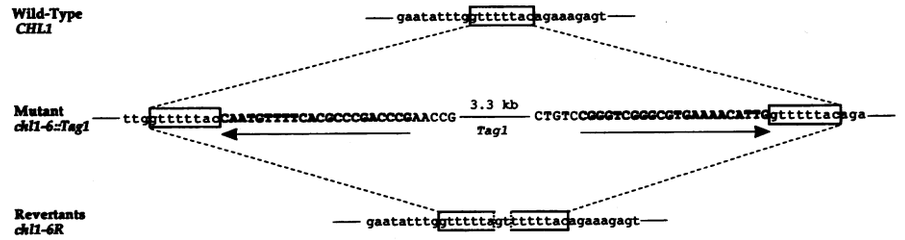

Fig. 2. Sequence of *Tag1* insertion in *chl1-6* mutant. DNA sequence of the target site of integration in the *CHL1* wild-type gene (top line), of the *Tag1-CHL1* junctions in the *chl1-6::Tag1* mutant (middle line), and of the excision site of four revertants, all of which had the same sequence (bottom line). *Tag1* sequences are in uppercase and adjacent sequences are in lowercase. Duplicated target sites are boxed. *Tag1* inverted repeats are in bold type and underlined by arrows. Sequence of *Tag1* element has been deposited in the GenBank Nucleotide Sequence Database under the accession number L12220.

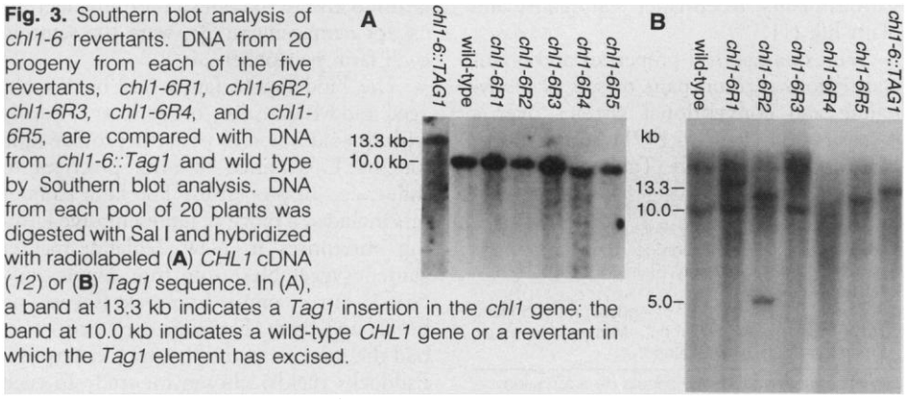

Fig. 3. Southern blot analysis of *chl1-6* revertants. DNA from 20 progeny from each of the five revertants, *chl1-6R1*, *chl1-6R2*, *chl1-6R3*, *chl1-6R4*, and *chl1-6R5*, are compared with DNA from *chl1-6::Tag1* and wild type by Southern blot analysis. DNA from each pool of 20 plants was digested with Sal I and hybridized with radiolabeled (**A**) *CHL1* cDNA (*12*) or (**B**) *Tag1* sequence. In (A), a band at 13.3 kb indicates a *Tag1* insertion in the *chl1* gene; the band at 10.0 kb indicates a wild-type *CHL1* gene or a revertant in which the *Tag1* element has excised.

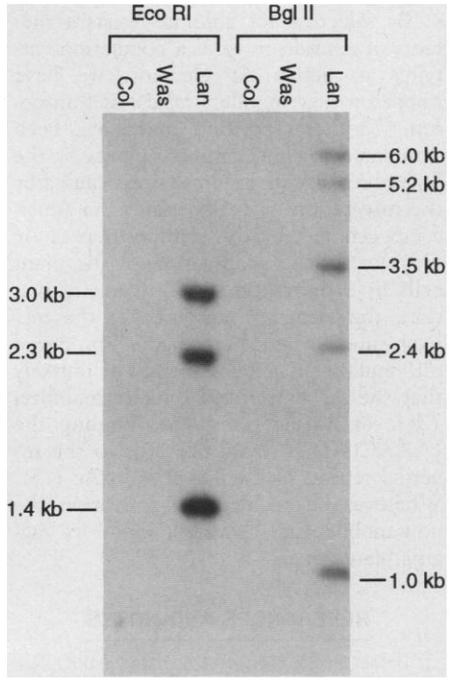

Fig. 4. Southern blot analysis of *Tag1* sequences in different ecotypes of *Arabidopsis*. DNA from ecotypes Landsberg *erecta* (Lan, the parental line containing the *erecta* mutation used for generating the *chl1-6* mutant), Columbia (Col), and Wassilewskija (Was) were digested with Eco RI or Bgl II as indicated and hybridized with a radiolabeled 1.4-kb Eco RI fragment from *Tag1* (see Fig. 1B). One Eco RI fragment (1.4 kb) and two Bgl II fragments (1 kb and one larger than 1.8 kb) from the *Tag1* element itself should hybridized to the probe; other bands are interpreted to originate from *Tag1*-related elements in Landsberg *erecta*.

locus, regenerating a 10-kb Sal I fragment of the *CHL1* gene, the same size found in wild-type plants (Fig. 3A). Sequence analysis of the *CHL1* gene in four of the revertants verified that the element had excised, leaving behind a small insertion (Fig. 2). In addition, new restriction fragments that hybridized with radiolabeled *Tag1* sequences were evident in the revertants (Fig. 3B). Thus, in the revertants, *Tag1* or *Tag1*-related elements had inserted into new loci. We conclude that *Tag1* is a mobile transposable element.

To confirm that *Tag1* is an endogenous element of *Arabidopsis*, genomic DNA was isolated from the untransformed parent used to construct the transgenic *Ac* lines. The parent originated from the ecotype Landsberg and carries the morphological mutation *erecta*. Southern blot analysis with radiolabeled *Tag1* DNA indicated that the Landsberg *erecta* parent contains *Tag1* and two additional *Tag1*-related elements, each present in only one copy per haploid genome (Fig. 4). No *Tag1* or related sequences were found in two other ecotypes of *Arabidopsis*, Columbia and Wassilewskija (Fig. 4).

By selecting for chlorate-resistant mutants of *Arabidopsis* from a population carrying an active *Ac* element, we have trapped a new mobile *Arabidopsis* transposon. *Tag1* transposition may have been stimulated in the Landsberg plants by the DNA breakage or genomic stress caused by the integration of T-DNA into the *Arabidopsis* genome, by the transposition of *Ac* (*13*), or by the propagation of the plant cells in tissue culture (*14*). Upon activation, the element transposed to the *chl1* locus and, when homozygous, produced *chl1* mutant progeny. We think it unlikely that the *Ac* transposase directly mobilizes *Tag1*, as no *Ac* transposase binding site (AAACGG) is found adjacent to the inverted repeats of *Tag1* as it is in *Ac* (*15*). Whatever the mechanism of activation, the now mobile *Tag1* should be useful for tagging plant genes.

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Soil Quality and Financial Performance of Biodynamic and Conventional Farms in New Zealand

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Biodynamic farming practices and systems show promise in mitigating some of the detrimental effects of chemical-dependent, conventional agriculture on the environment. The physical, biological, and chemical soil properties and economic profitability of adjacent, commercial biodynamic and conventional farms (16 total) in New Zealand were compared. The biodynamic farms in the study had better soil quality than the neighboring conventional farms and were just as financially viable on a per hectare basis.

Concerns about environmental, economic, and social impacts of chemical or conventional agriculture have led many farmers and consumers to seek alternative practices that will make agriculture more sustainable. Both organic and biodynamic farmers use no synthetic chemical fertilizers or pesticides, use compost additions and manures to improve soil quality, control pests naturally, rotate crops, and diversify crops and livestock. Unlike organic farmers, biodynamic farmers add eight specific preparations, made from cow manure, silica, and various plants, to enhance soil quality and plant life (*1*).

We examined soil properties and financial performance on pairs or sets of biodynamic and conventional systems over a 4-year period (1987 to 1991) on the North Island of New Zealand (Table 1). We also made financial comparisons between these farms and representative conventional farms in each study region on the basis of models used by the New Zealand Ministry of Agriculture and Fisheries (MAF) (*2*). A farm pair consisted of two side-by-side farms, one biodynamic and one conventional; a farm set consisted of three adjacent farms, one biodynamic and two conventional. The choice of five farm pairs and two farm sets (totaling 16 farms) was made on the basis of surveys, interviews, and on-farm soil examinations of more than 60 farms to ensure that all soil-forming factors, except management (*3*), were the same in each farm pair or set.

The biodynamic farms had been managed biodynamically for at least 8 years, with the oldest for 18 years, to provide time for the biodynamic farming practices to influence soil properties. The farm pairs or sets included a range of representative farming enterprises in New Zealand: market garden (vegetables), pip fruit (apples and pears), citrus, grain, livestock (sheep and beef), and dairy. Farms in each pair or set had the same crop and livestock enterprise. Paddocks (fields) chosen for study in each

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