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LETTER TO THE EDITOR

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Two transposable element insertions are causative mutations for the major domestication gene *teosinte branched 1* in modern maize

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Dear Editor,

Transposable elements (TEs) have long been regarded as genomic parasites and occupy a significant portion of eukaryotic genomes because of their massive replication in host genomes. However, at times, TEs can also be domesticated by genes and thereby contribute new regulatory functions to genes [1, 2]. Here we show that two TE insertions in a distant upstream region of *tb1* are the causative mutations in the *teosinte branched 1* (*tb1*) gene that account for the gene's role in the domestication of modern corn from its ancestor teosinte.

Modern cultivated maize was domesticated from its wild ancestor teosinte (*Zea mays* ssp. Parviglumis) about 10 000 years ago [3]. The plant morphological differences between cultivated maize and teosinte are dramatic. Such dramatic morphological changes are largely controlled by a small number of quantitative trait loci (QTLs). One of these loci is largely responsible for the plant architecture changes from wild teosinte to cultivated modern maize and is allelic to the recessive maize mutant *teosinte branched 1* (*tb1*). *tb1* was cloned by transposon tagging in maize [4]. It encodes a TCP transcriptional factor that is expressed in the axillary meristems and the stamen primordia of the maize ear [5]. It has been proposed that Tb1 could repress bud outgrowth and increase apical dominance. However, the difference between the maize and teosinte *tb1* alleles was quantitative, not qualitative. Both alleles may have nearly identical transcriptional units [6], but the maize allele has about twice the mRNA expression level of the teosinte allele. The *cis*-regulatory region that confers such molecular and phenotypic changes has been localized to a region 58-69 kb upstream of the *tb1* coding region [7]. This region is complex, containing two functional components within 64-69 kb and 58-64 kb, controlling basal branching and ear phenotypes, respectively [7]. However, the causative mutations contributing to the role of maize *tb1* in domestication are still unknown.

The *tb1* region of maize inbred line B73 was characterized previously (accession number: AF464738) [8]. We constructed bacterial artificial chromosome (BAC) libraries for teosinte (*Zea mays* ssp. Parviglumis, PI384061) and two maize inbred lines (Yu87-1 and W22). BAC clones containing the *tb1* region were isolated and sequenced (Supplementary information, Data S1).

A comparative analysis indicated that a long stretch of sequences surrounding the *tb1* coding region is highly conserved between maize and teosinte haplotypes (Figure 1). From 58 kb upstream to 5 kb downstream (the end of the sequenced maize BAC clones), the teosinte and maize haplotypes are highly conserved, except for an LTR retrotransposon inserted in maize haplotype about 13 kb upstream of the *tb1* coding region.

At 58-69 kb upstream is a critical region previously identified as the causal region for the *cis*-regulatory element changes to *tb1* during maize domestication [7]. Three major polymorphisms were found in this region, including a 4 884-bp copia-type LTR retrotransposon inserted in the maize haplotype at 58 858 bp upstream, a 379-bp miniature inverted-repeat transposable element (MITE) inserted in the maize haplotype at 64 461 bp upstream, and a 200-bp insertion present in the teosinte haplotype. The LTR retrotransposon has 227-bp LTRs and shares 99% identity (with only a 2-nucleotide difference), indicating that it was inserted recently. Further beyond 69 kb upstream, the sequence conservation started to drop dramatically, not only between the teosinte and maize haplotypes but also between maize haplotypes (Figure 1).

We further examined the 58-69 kb upstream region in detail by sequence analysis of additional maize and teosinte lines (Supplementary information, Figures S1 and S2). Sequence comparison of these maize and teosinte lines indicated that, except for the two TEs mentioned above, all other sequence polymorphisms failed to show an association with the maize lines and therefore were

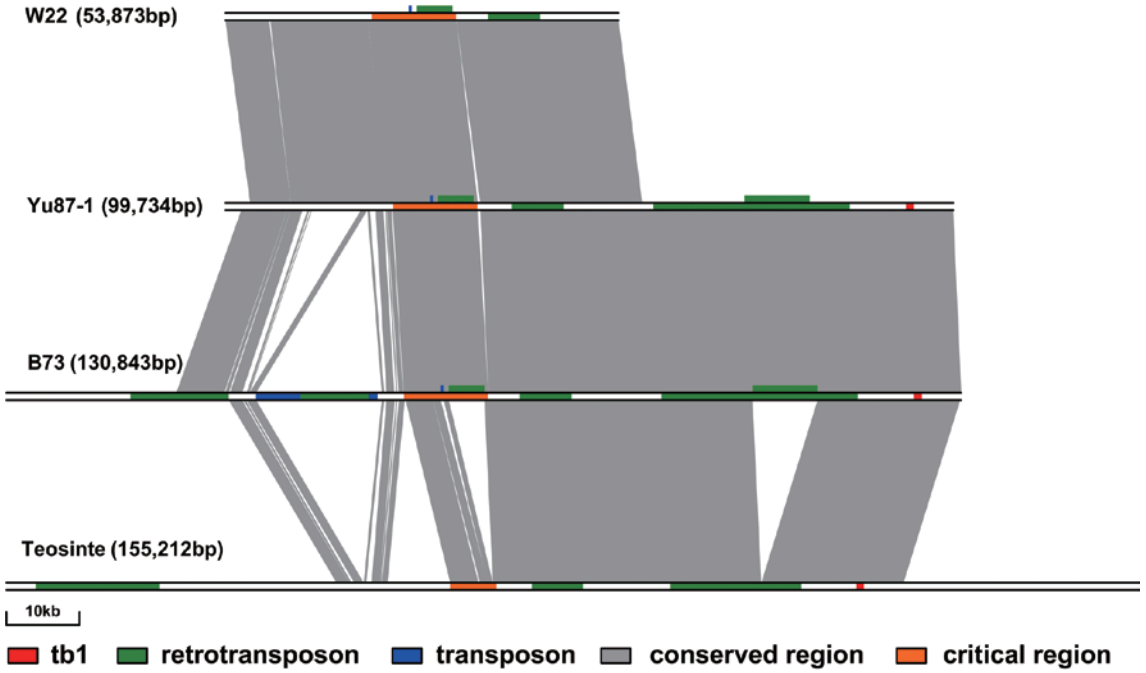

Figure 1 Sequence alignment of tb1 region from maize inbred lines B73, W22, Yu87-1. and Zea mays ssp. Parviglumis (PI 384061). Grey areas indicate homologous regions. Green bars indicate retrotransposon and blue bars indicate DNA transposons. Tb1 gene is indicated by red bars. Regulatory region of tb1 is indicated by orange bars. Insertion polymorphism of TEs between maize and teosinte are highlighted. All sequences are drawn to scale.

disqualified as the causative mutations for the maize *tb1* allele. The two TEs in the maize haplotypes appeared to be the only polymorphisms that could potentially be the causative mutations for the maize *tb1* allele.

To test this hypothesis, a maize association panel with 539 diverse lines (Supplementary information, Data S1, Table S1) and an extensive teosinte collection with 189 accessions were used for further association analysis (Supplementary information, Data S1, Table S2).

PCR products corresponding to the LTR retrotransposon insertion were detected in all 539 maize lines. Of these lines, 498 were detected using a primer set spanning the entire retrotransposon, whereas 41 were detected by a primer set bridging one end of the retrotransposon and the adjacent sequence (Supplementary information, Figure S3, Table S1). In teosinte, 174 of 189 accessions yielded PCR products without the TE insertion. Fourteen accessions were heterozygous for PCR products with both maize and teosinte haplotypes (Supplementary information, Figure S3, Table S2). One accession failed to yield any amplification product with the different primer sets.

All 539 maize lines showed a predicted 660-bp product corresponding to the MITE insertion. In teosinte, 163 of 189 accessions showed products lacking the MITE insertion. The remaining 26 accessions were heterozygous for the PCR products, with both maize and teosinte haplotypes (Supplementary information, Figure S3, Tables S1 and S2). In summary, both the LTR retrotransposon and the MITE showed exclusive association with all tested maize lines.

A previous genetic study revealed that the causative mutations for domestication in maize *tb1* are contained within a region located 58-69 kb upstream [7]. Our comparative genomics and association analysis revealed that the two TEs are the only polymorphisms associated with domesticated maize lines in this region. The exclusive association of the TEs with domesticated maize lines is in consistent with the strong selection for the domesticated trait controlled by the maize *tb1* gene. The maize domestication process selected for single-stalked maize against multi-tillered teosinte; this trait is controlled by the single locus *tb1*, and the maize tb1 allele behaves as dominant to the teosinte allele. The selection for the single-stalked trait was apparent and unambiguous, suggesting that the trait was under perfect selection during maize domestication.

Previous characterization of the 58-69 kb upstream region indicated the existence of two independent functional components [7]. These two independent compo-

nents, located 64-69 kb and 58-64 kb upstream of the maize tb1 allele, control maize basal branching and ear phenotype changes, respectively. The insertion positions of the two TEs correspond with these two functional components. The two TEs are the only maize-associated mutations within these two components, so they should be responsible for the functional changes. The observed functions of these two regulatory components also indicate that the insertions of these two TEs did not disrupt the preexisting regulatory functions (losses of function) but rather added new regulatory functions (gains of function). These gains of function are consistent with the genetic characteristics of the maize *tb1* allele as being dominant to the teosinte allele. Because they contributed new regulatory functions to the targeted gene, these two TEs are recognized as "domesticated" or "exapted" [1].

According to the previous functional characterization [7], the observed function of the 64-69 kb upstream component in maize is to suppress basal branching. Based on the biological function of the *tb1* gene [4], we predict that the mutation in this component may confer a quantitative increase in the expression of the maize *tb1* allele. This increased expression is consistent with the potential effect of the MITE inserted within this component. It is well documented that MITEs inserted upstream of a gene often result in up-regulated gene expression [9]. Because the 58-64 kb upstream component affected the ear phenotype in maize, we predict that the mutation in this component may confer a change in the tissue specificity of *tb1* gene expression. It is known that LTR retrotransposon insertion can change the tissue-specific expression of an adjacent gene [10]. Therefore, the LTR retrotransposon inserted in the 58-64 kb component may be responsible for the change in tissue specificity of the maize *tb1* allele. Of course, the predicted functions of these TEs require further experimental verification.

We also observed a limited distribution of haplotypes with these two TEs among the teosinte accessions (26 accessions for the MITE and 14 accessions for the LTR retrotransposon). These accessions all belonged to the subspecies Parviglumis and Mexicana, the two subspecies closest to cultivated maize. The presence of these TEs among teosinte accessions cannot simply be explained by cross-contamination of maize haplotypes with teosinte accessions. Only 7 accessions contained possible maize haplotype contamination with both the MITE and the LTR retrotransposon. The majority had either the MITE (19 accessions) or the LTR retrotransposon (7 accessions). We propose that the MITE and LTR resulted from two independent transpositions in teosinte that predated maize domestication and that these two TEs were coupled through hybridization and recombination between different teosinte haplotypes harboring one of the two TEs. Then the domestication selection fixed both TEs in modern maize haplotypes.

The change in the regulation of the *tb1* gene during maize domestication was subtle and sophisticated [4, 7]. It is difficult to achieve such a change by accumulating only point mutations. However, TEs containing one or more *cis*-regulatory elements can move to a new location by a single transposition and thereby add new regulatory functions to the targeted gene, speeding up a process that could have required million years of evolution without the TE. In this manner, TEs could greatly accelerate the evolution of the host genome in response to sudden challenges [11]. If the host were to encounter a critical crisis, reactivation of many silenced TEs stored in the genome may enhance the host's chance of survival by quickly evolving new gene-regulatory functions in a short time period. In view of the great abundance of TEs and their global distribution within eukaryotic genomes, the impact of TEs and their activities on the evolution of gene regulation and function could exceed our previous estimates. Such mechanisms involving TEs could theoretically explain some of the extraordinary biological diversity observed in nature and could provide new opportunities for genetic breeding with exhausted genetic resources.

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(**Supplementary information** is linked to the online version of the paper on the *Cell Research* website.)