Tuned for Transposition: Molecular Determinants Underlying the Hyperactivity of a Stowaway MITE

Guojun Yang,1,2,3 Dawn Holligan Nagel,1 Cédric Feschotte,4 C. Nathan Hancock,1 Susan R. Wessler1*

Miniature inverted repeat transposable elements (MITEs) are widespread in eukaryotic genomes, where they can attain high copy numbers despite a lack of coding capacity. However, little is known about how they originate and amplify. We performed a genome-wide screen of functional interactions between Stowaway MITEs and potential transposases in the rice genome and identified a transpositionally active MITE that possesses key properties that enhance transposition. Although not directly related to its autonomous element, the MITE has less affinity for the transposase than the autonomous element but lacks a motif repressing transposition in the autonomous element. The MITE contains internal sequences that enhance transposition. These findings suggest that MITEs achieve high transposition activity by scavenging transposases encoded by distantly related and self-restrained autonomous elements.

Most eukaryotic genomes contain large numbers of many different types of transposable elements (TEs). The vast majority of these TEs cannot replicate and mobilize themselves into new regions of the genome (they are nonautonomous). These elements thus depend on transposases encoded by other, autonomous, elements. Miniature inverted repeat TE (MITEs) are a type of nonautonomous element found in both prokaryotic and eukaryotic genomes, where they are often located in or near genes (1–3). MITEs resemble typical DNA transposons, although they tend to have a small size (<500 base pairs (bp)) and high copy number and contain terminal inverted repeats (TIRs) flanked by target site duplications. Although MITEs lack coding capacity, in plants they are classified as either Tourist-like or Stowaway-like (4). Although the high copy numbers of MITEs in plant genomes suggest that they have high transposition activity, only the Tourist-like rice element mPing, an internal deletion derivative of its autonomous partner Ping, has been shown to be currently active (5–7). However, the mechanism by which rice strains accumulate 100- to 1000-fold more mPing than Ping elements is unknown (8).

Unlike mPing, the vast majority of characterized MITEs are not deletion derivatives of existing autonomous transposons (9, 10). Furthermore, because none of these MITEs are active, their origin, success, and source of transposase have been a mystery. The most logical model to explain the movement of these nondeletion derivative MITEs is that they can borrow the transposase of distantly related elements (in a process referred to as cross-mobilization) and amplify within the genome (4).

The sequenced genome of Oryza sativa (rice) contains more than 22,000 Stowaway MITEs belonging to at least 25 subfamilies (10). Furthermore, the structure of MITE phylogenetic trees indicates that subfamilies are derived from the amplification of one or a few individual elements to hundreds or thousands of copies (10). Stowaway elements have not previously been shown to be active in rice or in any other genome. Surprisingly, none of the Stowaway families appeared to be deletion derivatives of any transposase-encoding element found within sequenced rice genomes. It was predicted that rice Mariner-like elements (called Osmars) were the most likely source of transposase because Stowaway and Osma share...
terminal inverted repeats (of ~10 bp) and the same target site duplication (the dinucleotide TA) (Fig. S1).

On the basis of their transposase protein sequences, Osmars were classified into 25 families and placed in three major clades (10). Among the 34 Osmars, only five contain intact transposase-coding regions. Here, we refer to Osmars with complete ends and intact coding sequences as potentially autonomous elements, those with mutated or truncated coding sequences as nonautonomous elements, and small elements lacking any coding sequences as deletion derivatives of Osmar (Fig. S1).

To determine whether any Osmar transposases could catalyze the transposition of rice Stowaway MITEs, we modified a yeast assay previously developed to demonstrate transposition of a nonautonomous version of Osmar5 (OsmNA) by its own transposase (11). The assay has two plasmid components (Fig. 1): One is a transposase (Tpase) source that expresses one of seven Osmar transposases (abbreviated Osm1, Osm5...) representing each Osmar subclade under the control of an inducible yeast promoter (Pgpd1) (12). The second plasmid in our assay contains the ade2 reporter gene disrupted by one of 24 nonautonomous elements, including 17 Stowaway (abbreviated Osm5, Osm8...) chosen to represent the diversity of Stowaway families in rice, and seven direct-deletion derivatives of each Osmar element (abbreviated OsmXNA, where NA means nonautonomous) (12). In yeast cells containing both plasmids, potentially successful transposase-transposon interactions were scored on the basis of Ade2 revertant colonies (Fig. 1).

These tests revealed that six of the seven transposases showed evidence of activity and uncovered several instances of cross-mobilization (Fig. 1 and table S1). The nonautonomous versions of Osmar1 and Osmar5 (Osm1NA and Osm5NA) were excised in the presence of their cognate transposases, and cross-mobilization occurred for Osm17NA by Osm5; Osm19NA by Osm9, Osm10, and Osm14; and Osm14NA by Osm9. We also identified interactions between three Stowaway elements and Osmar transposases: Osm8 by Osm1 and Osm17, and Osm16 and Osm35 by Osm14 (Fig. 1 and table S1). In contrast to the low excision activities observed for Osm8 and Osm16, the excision of Osm35 by Osm14 was the highest interaction recorded by this assay (Fig. 1 and fig. S2A). Polymerase chain reaction (PCR) amplification confirmed independent excision events of Osm35 by the Osmar14 transposase (fig. S2B), and sequencing of the PCR products revealed excision footprints (fig. S2C) similar to those retrieved for Osmar5 in this and a previous study (fig. S3A) (11). Additionally, reinsertions of Ost35 into chromosomal loci were confirmed by means of Southern hybridization of yeast genomic DNA (figs. S2D and S3B). The fact that Stowaways were mobilized by Osmar transposases provides a functional mechanism for the mobilization of Stowaway MITEs in the rice genome. The observed excision of one Stowaway by two distinct transposases and the excision of two different Stowaways by the same transposase suggest that cross-mobilization may be a major mechanism for the amplification of rice MITEs.

The differences in excision frequency between Ost35 and Osm14NA mirrored the difference in copy number between Stowaway MITEs and Osmar elements in the rice genomes (10). Several properties of the MITEs could explain the differential activity of Ost35 as compared with Osm14NA.

First, it is possible that the differences in size between Osm14NA and Ost35 (1004 versus 239 bp) may be a factor because increases in TE size has been shown to reduce transposition efficiency (13, 14). Second, because transposition of Mariner elements requires the binding of transposase to transposon TIRs and/or subterminal regions to form synaptic complexes for subsequent excision and reintegration (15), any differential binding of Osmar14 transposase to the ends of Osm14NA and Ost35 may have affected their transposition activity. Ost35 has shorter TIRs than Osmar14 (20 versus 32 bp) and different subterminal sequences. Finally, Ost35 and Osm14NA also differ in their internal sequences, which may enhance or repress excision.

To investigate whether element size affects excision frequency, we shortened Osm14NA (1004 bp) to the length of Ost35 (239 bp) (now called Osm14NAS) (Fig. 2 and fig. S4). Relative to Osm14NA, the shorter Osm14NAS displayed weak but detectable excision activity (Fig. 2). Thus, size alone cannot account for the magnitude of excision activity of Ost35.

To investigate how transposase binding differed between elements, we examined the interaction of purified recombinant Osmar14 transposase with synthesized oligonucleotides corresponding to the terminal (32 bp) and subterminal (32 bp) regions of Ost35 and Osm14NA (Fig. 3A and fig. S4) in electrophoretic mobility shift assays. These experiments revealed differences in transposase affinity but not in the expected direction. For Ost35, the 5' and 3' terminal regions (abbreviated 5'T and 3'T, respectively, in Fig. 3A and fig. S4) each bound transposase equivalently. There was also some binding, albeit less efficiently, to each of the subterminal regions of Ost35 (5' subT and 3' subT) of Ost35 by the Osmar14 transposase (Fig. 3A and fig. S4) in electrophoretic mobility shift assays. These experiments revealed differences in transposase affinity but not in the expected direction. For Ost35, the 5' and 3' terminal regions (abbreviated 5'T and 3'T, respectively, in Fig. 3A and fig. S4) each bound transposase equivalently. There was also some binding, albeit less efficiently, to each of the subterminal regions of Ost35 (5' subT and 3' subT) of Ost35 by the Osmar14 transposase (Fig. 3A and fig. S4) in electrophoretic mobility shift assays. These experiments revealed differences in transposase affinity but not in the expected direction. For Ost35, the 5' and 3' terminal regions (abbreviated 5'T and 3'T, respectively, in Fig. 3A and fig. S4) each bound transposase equivalently. There was also some binding, albeit less efficiently, to each of the subterminal regions of Ost35 (5' subT and 3' subT) of Ost35 by the Osmar14 transposase (Fig. 3A and fig. S4) in electrophoretic mobility shift assays. These experiments revealed differences in transposase affinity but not in the expected direction. For Ost35, the 5' and 3' terminal regions (abbreviated 5'T and 3'T, respectively, in Fig. 3A and fig. S4) each bound transposase equivalently. There was also some binding, albeit less efficiently, to each of the subterminal regions of Ost35 (5' subT and 3' subT) of Ost35 by the Osmar14 transposase (Fig. 3A and fig. S4) in electrophoretic mobility shift assays. These experiments revealed differences in transposase affinity but not in the expected direction. For Ost35, the 5' and 3' terminal regions (abbreviated 5'T and 3'T, respectively, in Fig. 3A and fig. S4) each bound transposase equivalently. There was also some binding, albeit less efficiently, to each of the subterminal regions of Ost35 (5' subT and 3' subT) of Ost35 by the Osmar14 transposase (Fig. 3A and fig. S4) in electrophoretic mobility shift assays. These experiments revealed differences in transposase affinity but not in the expected direction. For Ost35, the 5' and 3' terminal regions (abbreviated 5'T and 3'T, respectively, in Fig. 3A and fig. S4) each bound transposase equivalently. There was also some binding, albeit less efficiently, to each of the subterminal regions of Ost35 (5' subT and 3' subT) of Ost35 by the Osmar14 transposase (Fig. 3A and fig. S4) in electrophoretic mobility shift assays. These experiments revealed differences in transposase affinity but not in the expected direction. For Ost35, the 5' and 3' terminal regions (abbreviated 5'T and 3'T, respectively, in Fig. 3A and fig. S4) each bound transposase equivalently. There was also some binding, albeit less efficiently, to each of the subterminal regions of Ost35 (5' subT and 3' subT) of Ost35 by the Osmar14 transposase (Fig. 3A and fig. S4) in electrophoretic mobility shift assays. These experiments revealed differences in transposase affinity but not in the expected direction. For Ost35, the 5' and 3' terminal regions (abbreviated 5'T and 3'T, respectively, in Fig. 3A and fig. S4) each bound transposase equivalently. There was also some binding, albeit less efficiently, to each of the subterminal regions of Ost35 (5' subT and 3' subT) of Ost35 by the Osmar14 transposase (Fig. 3A and fig. S4) in electrophoretic mobility shift assays. These experiments revealed differences in transposase affinity but not in the expected direction. For Ost35, the 5' and 3' terminal regions (abbreviated 5'T and 3'T, respectively, in Fig. 3A and fig. S4) each bound transposase equivalently. There was also some binding, albeit less efficiently, to each of the subterminal regions of Ost35 (5' subT and 3' subT) of Ost35 by the Osmar14 transposase (Fig. 3A and fig. S4) in electrophoretic mobility shift assays. These experiments revealed differences in transposase affinity but not in the expected direction. For Ost35, the 5' and 3' terminal regions (abbreviated 5'T and 3'T, respectively, in Fig. 3A and fig. S4) each bound transposase equivalently. There was also some binding, albeit less efficiently, to each of the subterminal regions of Ost35 (5' subT and 3' subT)
the 3′ subT in Fig. 3A and fig. S4). In contrast, both the terminal and subterminal regions of Osmar14 bound to transposase more strongly than the equivalent regions in Ost35. In addition, binding to Osmar14 was asymmetrical because sequences from the 3′ end of the element showed more binding affinity than those from the 5′ end.

We detected no binding to the sequences internal to the subTs of either Ost35 or Osm14NA (Fig. 3, B and C, and fig. S4). Furthermore, when unlabeled Osm14NAS or Ost35 was used to compete with radiolabeled Osm14NAS, Ost35 did not outcompete Osm14NAS for transposase binding (fig. S5). These results suggest that the higher excision frequency of Ost35 is not due to an increased affinity for the Osmar14 transposase.

We swapped multiple regions between Osm14NAS and Ost35 and confirmed that the central region of Ost35 is involved in enhancing the frequency of excision (Fig. 2). However, this experiment showed, surprisingly, that despite the poor function of Osm14NAS in the excision assay, the replacement of the Ost35 TIRs by those of Osmar14 (a chimeric element we called 14T32) resulted in a ~25-fold increase in excision as compared with the original Ost35 transposon or a modified Ost35 with a different internal sequence of the same length (in this case, derived from the gfp gene). These data suggest that the function of the TIRs is normally suppressed in Osm14NAS (and also presumably in the natural Osmar14 element), whereas the function of the Ost35 TIRs is enhanced by the sequence between them. Further supporting this theory, we observed that the subTs of Osm14NAS represses excision when combined with either the Osmar14 TIRs, the equivalent regions of Ost35, or a different internal sequence (Fig. 2). Also, the region of Ost35 between the terminal sequences (32 bp) enhanced excision of adjacent TIRs by ~20-fold relative to the random internal sequence.

Taken together, these data support two general conclusions. For Osm14NAS, the extremely low excision frequency suggests that there is repression of the otherwise optimal Osmar14 TIRs by one or both of its subterminal regions. For Ost35, the high excision frequency most likely indicates that there is enhancement of the otherwise suboptimal Ost35 TIRs by part of or the entire Ost35 internal region.

We next performed site-directed mutagenesis so as to more precisely localize the regions responsible for the enhancement and repression of excision in the internal region of Ost35 and the Osm14NAS subTs, respectively. We used 14T32 and Osm14NAS constructs as the templates for mutagenesis. For each construct, we replaced consecutive blocks of eight nucleotides by the sequence ATTTAAAT (SwaI restriction site), resulting in 22 derivative constructs from each starting template. Among the mutant constructs derived from 14T32, a 75 to 80% decrease in excision activity was caused by mutations distributed throughout the Ost35 internal region, whereas a single mutation showed an approximately three-
Reports

The RNA-Binding Protein NANOS2 Is Required to Maintain Murine Spermatogonial Stem Cells

Aiko Sada,1 Atsushi Suzuki,2 Hitomi Suzuki,3 Yumiko Saga1,3,4*

Stem cells give rise to differentiated cell types but also preserve their undifferentiated state through cell self-renewal. With the use of transgenic mice, we found that the RNA-binding protein NANOS2 is essential for maintaining spermatogonial stem cells. Lineage-tracing analyses revealed that undifferentiated spermatagonia expressing Nanos2 self-renew and generate the entire spermatogonial cell lineage. Conditional disruption of postnatal Nanos2 depleted spermatogonial stem cell reserves, whereas mouse testes in which Nanos2 had been overexpressed accumulated spermatagonia with undifferentiated, stem cell–like properties. Thus, NANOS2 is a key stem cell regulator that is expressed in self-renewing spermatogonial stem cells and maintains the stem cell state during murine spermatogenesis.

S
tem cells are essential for tissue homeostasis and regenerative responses to injury and disease. In the spermatogenic stem cell system, germ cell–intrinsic factors have an essential role in the maintenance of stem cells for the continuation of spermatogenesis throughout life (1–5). However, the previous loss-of-function studies have some limitations in terms of understanding the mechanism by which stem cells are lost upon the gene deletion, as it could be caused by cell death, defective self-renewal, premature differentiation, or other mechanisms.

For decades, the mammalian spermatogonial stem cell has been characterized by the morphological features of the spermatogonia. The spermatogonial types \(A_{\text{single}}\) (\(A_s\), isolated single cells), \(A_{\text{paired}}\) (\(A_p\), chains of 2 cells), and \(A_{\text{aligned}}\) (\(A_a\), chains of 4, 8, 16 or 32 cells) are the most primitive germ cells observed in mature testes and are collectively described as undifferentiated spermatagonia. They give rise to differentiating spermatagonia, which undergo additional divisions and enter a differentiation pathway. It has been proposed that only \(A_s\) spermatagonia represent the stem cells (6–8); however, there is no \(A_s\)-specific molecular marker, and the presence of stem cells is assayed by long-term colony formation after the transplantation of candidate cells into recipient testes (9). For this reason, undifferentiated spermatagonia containing \(A_s\) to \(A_a\) are the smallest population proven to have the properties of stem cells.

Recently, two functionally distinct spermatogonial stem cell populations were identified in mice (10). One is the population that acts as the self-renewing stem cells (actual stem cells), and the other population possesses the potential to self-

References and Notes


12. Materials and methods are available as supporting material on Science Online.
21. We thank C. F. Weil and D. J. Garfinkel for materials and technical assistance and J. Bennetzen, X. Zhang, and J. Leebers-Mack for discussion and insightful comments. This work was supported by NIH, the NSF Plant Genome Program, the University of Georgia Research Foundation, and the University of Toronto. Sequences for Osma transposase coding sequences and their nonautonomous elements were deposited in GenBank (accession numbers GQ379705 to GQ379718 and GQ382183).

Supporting Online Material
www.sciencemag.org/cgi/content/full/325/5946/1391/DC1
Materials and Methods
Figs. S1 to S5
Table S1
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Sequences and Primers
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Supporting Online Material for

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Methods and Materials
Plasmid construction and yeast excision assay
Exons of Osmar coding sequences, as determined by the alignment of Osmar transposase sequences, were amplified from rice genomic DNA (Osmar5, Osmar9, Osmar10, Osmar14) or BAC/PAC vectors (Osmar1, Osmar17, Osmar19 from CUGI clone OSJNa0051P13, OSJNb0024B16 and OSJNa0050F15 respectively, Clemson University Genomics Institute, Clemson, SC) before they were joined together with PCR via an overlapping region on the primers to obtain full length coding sequences. The single nucleotide deletion mutation close to the end of the first exon of Osmar14 was repaired based on the alignment of Osmar transposases using a PCR primer with the corrected sequence. The coding sequences for Osmar5 and Osmar10 were obtained as previously described (S1). Nonautonomous versions of the Osmars that served as transposase source were constructed by joining the fragments of ~450 bp from each end by PCR. Osm14NA (1004 bp) was further shortened to the size of Ost35 (239 bp) by a similar approach, resulting in Osm14NAS element. Because the 5’ end of Osmar19 is missing in the sequenced rice genome, Osm19NA was generated with identical but inverted 5’ and 3’ ends. The Stowaway elements used in this study were chosen on the basis of their family diversity and then similarity to the consensus sequences of corresponding families was used. Stowaways were first amplified from rice genomic DNA with PCR primers corresponding to the flanking sequences of each element extracted from rice genome sequence with a MITE Analysis Kit (MAK)(S2). Individual Stowaway sequences were subsequently amplified with element specific primers.

Transposase coding sequences were cloned into a pRS413 based vector, as described (S3). The nonautonomous Osmars and Stowaways were cloned into the HpaI site inside the ade2 reporter gene on pWL89A (S4). Excision assays were performed, as described (S3). Genomic DNA of ADE2 revertants was used as template for PCR to confirm TE excision with primers flanking TE donor sites. Genomic DNA blot analysis was performed, as described (S3). Sequences of TE elements and primers are included in this document.

Electrophoretic mobility shift assay (EMSA)
The coding sequence of the Osmar14 transposase was fused to the maltose binding protein gene on expression vector pMal-c2x (NEB, Ipswich, MA), expressed in E.coli (BL21) and purified with amylose beads (NEB) as was the maltose binding protein control. While the short fragments (32 bp) used for Fig. 2A were synthesized and annealed before performing the assay, the internal sequences or those including subterminal sequences used for Fig. 2C were obtained by PCR using full length Osm14NAS and Ost35 as templates. The Osmar14 3’ subT fragment in Fig. 2A does not include six of the 12 bp low complexity poly C stretch between the 3’ subT and 3’ TIR. Double stranded DNA fragments were end labeled with P33 using T4 kinase (Invitrogen, Carlsbad, CA). Quantification of the intensity of the shifted bands was performed with Adobe Photoshop. The relative binding intensity was defined as the ratio of the signal intensity of the shifted band to that of the unbound DNA. Quantification was performed for three independent experiments.

Competition assay
Osm14NAS PCR products were labeled with P33 using T4 kinase (Invitrogen). Each reaction contained 15 ng of labeled DNA and 0.5 μg MBP (control) or Osmar14 TPase:MBP fusion protein in 15 mM Tris (pH 7.5), 0.1 mM EDTA, 1 mM DTT, 0.3
mg/ml BSA, 0.1% NP-40, 10% glycerol and 33 μg/ml single-stranded DNA. Unlabeled Osm14NAS and Ost35 PCR products were used for competition at the following concentrations: 75 ng, 150 ng, 300 ng. Samples were separated on a 4% native polyacrylamide gel (0.5x TBE) after a 1 hour incubation at room temperature.

Construction of chimeric elements
To obtain the chimeric constructs in Figure 3 (those that have a “T” in their name, e.g. 14T32), PCR primers were designed to contain the terminal 32 bp of one element (e.g. Ost35) as 5’ overhangs attached to the oligos priming into the subterminal region of the other element (e.g. Osm14NAS). Similarly, to obtain constructs bearing swapped terminal 65 bp regions, the 65 bp terminal sequences (e.g. of Ost35) were attached as 5’ overhangs to the oligos priming into the internal sequences of the other element (e.g. Osm14NAS). Chimeric PCR products were then cloned into the HpaI site in the ade2 reporter gene for use in the yeast excision assay. To construct control plasmids containing the terminal (32 or 65 bp) sequences of Osm14NAS or Ost35, a randomly chosen region in the green fluorescent protein gene (mGFP5-er) was used as template with chimeric primers designed in a similar way as that described for the construction of the chimeric versions of Ost35 and Osm14NAS.

Site-directed mutagenesis
For site-directed mutagenesis, primers (~45 nt) were designed to include ~18 nt on both sides of a mutation target site (8 nt), to be replaced with a SwaI site (ATTTAAAT). Mutagenesis was performed according to the manufacturer’s manual with a QuikChange Mutisite-directed mutagenesis kit (Stratagene, La Jolla, CA). Mutant constructs were identified by the presence of a SwaI site through restriction enzymatic digestion (SwaI and SalI) and/or sequencing. Primer sequences are included in this document.

Supplemental Figure Legends:

**Fig. S1.** Osmars and Stowaways in rice. (A) Approximate copy numbers of Osmar (autonomous and nonautonomous) and Stowaway elements in the sequenced rice genome (numbers in parenthesis) (Oryza sativa ssp. japonica cv nipponbare). The pie-chart is not drawn to scale as the proportion of Osmars is one tenth of that shown. (B) Relationship of autonomous Osmars, nonautonomous Osmars, deletion derivatives and Stowaways. CTCCCTCYGT is the terminal sequences shared among Osmars and Stowaways; black triangles represent TIRs; green regions in Osmars represent transposase genes with introns (vertical white bars); red stars indicate nonsense or frame-shifting mutations; colored Stowaways represents different families.

**Fig. S2.** (A) ADE2 revertants following excision of Ost35 catalyzed by the Osmar14 transposase. The tpase source is shown in white letters; Donor indicates the element inserted into ade2. Each plate contains three sectors representing three independent events. (B) PCR analysis of the donor sites in ADE2 revertants. C, plasmid control; PCR primer positions are shown in 1A. (C) DNA sequence of donor sites after excision of Ost35. The sequence of the donor site before excision is shown at the top and excision sites from independent ADE2 revertants are below. (D) Genomic DNA blot analysis of ADE2 revertants from Ost35 and 14T32. Yeast genomic DNA was digested with DraI and separated by agarose gel electrophoresis. Radioisotope (P32) labeled DNA fragment
corresponding to the sequence of Ost35 was used as probe. C, plasmid control that resulted in a single band of ~3 kb.

**Fig. S3.** Donor sites (A) and insertion sites (B) of OsmNAs and *Stowaways* after transposition. The donor sites for Ost35 are shown in Fig. 1D. Red letters, target site duplications. The insertion sites of *Osmar5* were previous determined (S3).

**Fig. S4.** Engineered internal deletion derivatives of *Osmar*14 and regions used for EMSA. Arrowheads, TIRs; vertical dotted lines, break points in Osm14NA and Osm14NAS. Regions used in EMSA shown in Fig. 2 are indicated for both Osm14NAS and Ost35.

**Fig. S5.** Competition assay between Osm14NAS and Ost35. Wedge, increasing amount of unlabeled DNA; Small arrowhead indicates where DNA was bound by transposase at one site; large arrowhead indicates where DNA was bound by transposase at multiple sites; lower band (no arrow), unbound labeled DNA. The entire Osm14NAS was end labeled with P33 radioisotope. Equal molar amounts of labeled DNA was added in each lane and equal molar amounts of unlabeled DNA was used in corresponding lanes of Osm14NAS and Ost35.

**Table S1. ADE2 reversion of nonautonomous Osmars and Stowaways**

<table>
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<th>Donor</th>
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The number of colonies on YPD media and revertants on media lacking adenine are shown only for the combinations that produced *ADE2* revertants. The cells in each galactose-induced colony were suspended in 50 μl of water and plated on media lacking adenine. An equal volume, but diluted 3.6x10^5 times from the aforementioned cell suspension, was plated on YPD media to obtain the total number of viable cells in the galactose-induced colony. Each number separated by a comma is the number of colonies/revertants grown from one galactose-induced colony. The number of *ADE2*
revertants is shown beneath that on YPD plate correspondingly. “-”, colonies too dense to get an accurate count (>500).

**Supplemental References:**
Sequences and Primers

1. Accession numbers for *Osmar* transposase CDS and nonautonomous elements

<table>
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<th>Transposase</th>
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<td><em>BamHI</em>, <em>XhoI</em></td>
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2. Accession numbers and positions for *Stowaways* used in this study

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<td>Ost8</td>
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<td>Ost10</td>
<td>BX548156</td>
<td>36272 to 36014</td>
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<td>Ost11</td>
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3. Primers for Osmar coding sequence cloning

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<th>Primer Name</th>
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<td>actatgatcatgcagctgtagtggttttgtttctcatttagaagatcgtagc</td>
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<td>osmar19-XhoI</td>
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<td>osmar14-BamHI</td>
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<td>osmar14-XhoI rev</td>
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<td>osmar14-exon2 rev</td>
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<td>osmar17-BamHI</td>
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<td>osmar17-EcoRI rev</td>
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<td>osmar17-exon2 rev</td>
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4. Primers for OsmNA cloning

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<tr>
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Osm19 middle short acctcactccgagaaggacccgagggtgcgaagttatacagc
Osm14NAS internal 5’ rev cttatattgtaataagttggtggttagattagtagtaatacagc
Osm14NAS internal 3’ for cctaattaggtacaaataaagltattatgtgccccacatagatgc

5. Primers for Stowaway cloning

First PCR (flanking)

Os10_flank for gaaaaataatttaggtaggtagatcaa
Os10_flank rev catgttttgaatgacatatcaca
Os11_flank for ttacaagtgctctagttcttttaac
Os11_flank rev caattgactaaggtctttatgttaa
Os13_flank for ggtagagattgtgtgctttttaa
Os13_flank rev gtaaagtgaatgaacataagtgtatct
Os14_flank for tcaaaaaattttgttagtgctcata
Os14_flank rev ggccaaacgcgacctttagatcaca
Os15_flank for gaaggccggcacaagatgttaattgtaa
Os15_flank rev agagacagctttggtcatactacg
Os16_flank for gcctggtactgtctgtgtgcaat
Os16_flank rev tgtagattcactataaactca
Os18_flank for tccaaatatataagcgttagttagc
Os18_flank rev cccaaatatataaatgctttatcaatcg
Os20_flank for ctttttttttttcacaaagaggg
Os20_flank rev gacacattctagtataaaatttag
Os24_flank for cacacattcttaatattctctac
Os24_flank rev gagggtgacggggaattaaattag
Os28_flank for gcgtcttttatttaatgactgataac
Os28_flank rev ggtgcctccccacaaccaacacctc
Os34_flank for ctgctataatctgctactttgat
Os34_flank rev gaatttttcatgatcaataaatag
Os35_flank for tacggttagcaaggacactgata
Os35_flank rev gcctctgtagaggtgctgattacc
Os42_flank for taattttggaatcatgcaatcag
Os42_flank rev ccagtagctcctcataattgacattt
Os46_flank for aaaaaagtaggtgggtattagtgacag
Os46_flank rev catgtttgctgacttttagctca
Os5_flank for agagagcctcaacccagttgcaac
Os5_flank rev gttggtttcattcgattcttattgac
Os52_flank for ggtgtgtaggtgctgataattac
Os52_flank rev ggtgttacaaagatgaccagaggta
Os8_flank for atcaggtttgacaaagattttgataaagaaatatat
Os8_flank rev attttgataatgatttttgatttagaaatag

Second PCR (TIR regions)

Os10_tir_for_code /5'phos/tacctctccgtactctgtaaaa
Os10_tir_rev_code /5'phos/tacctctccgtactctgtaaa
Os11_tir_for_code /5'phos/tacctctccctatttcactttaa
Os11_tir_rev_code /5'phos/tacctctccatctacttttga
Os13_tir_code /5’phos/tacctccctcatccccaaatat
Os14_tir_for_code /5’phos/tacctccctcatccccaaagtt
Os14_tir_rev_code /5’phos/tacctttctcatccccaaaggt
Os15_tir_code /5’phos/tacctccctcatccccaaatat
Os16_tir_for_code /5’phos/tacctccctgtcctccaaatat
Os16_tir_rev_code /5’phos/tacctccctgtcctccaaaggt
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Os8_tir_code /5’phos/tacctccctgtcctccaaatat

6. Primers for TIR swapping

Osm14NA TIR 5’-32-ost35intl /5’phos/tacctccctgtcctccaaatat
Ost35 TIR 3’-32-osm14intl /5’phos/tacctccctgtcctccaaatat
Ost35 TIR 5’-32-osm14intl /5’phos/tacctccctgtcctccaaatat
Osm14NA TIR 3’-32-ost35intl /5’phos/tacctccctgtcctccaaatat
Osm14NA TIR 5’-65-ost35intl /5’phos/tacctccctgtcctccaaatat
Ost35 TIR 5’-65-osm14intl /5’phos/tacctccctgtcctccaaatat
Osm14NA TIR 3’-65-ost35intl /5’phos/tacctccctgtcctccaaatat
Ost35 TIR 3’-65-osm14intl /5’phos/tacctccctgtcctccaaatat
Osm14NA TIR 3’-65-GFPintl1 /5’phos/tacctccctgtcctccaaatat
Ost35 TIR 3’-65-GFPintl1 /5’phos/tacctccctgtcctccaaatat
Osm14NA TIR 5’-32-GFPintl1 /5’phos/tacctccctgtcctccaaatat
Ost35 TIR 3’-32-GFPintl1 /5’phos/tacctccctgtcctccaaatat
Ost35 TIR 5’-65-GFPintl1 /5’phos/tacctccctgtcctccaaatat
Ost35 TIR 3’-65-GFPintl1 /5’phos/tacctccctgtcctccaaatat
7. Primers for site directed mutagenesis

7.1 14T32_int_mut1  cagaaagaagggattcctgtgatttaaatgtggagatgg
7.1 14T32_int_mut2  atacatctcgacatcccgatatttaaatgtggagatgg
7.1 14T32_int_mut3  ctgaatcctgcagatatttaaatgtggagatgg
7.1 14T32_int_mut4  cagaaagaagggattcctgtgatttaaatgtggagatgg
7.1 14T32_int_mut5  cagaaagaagggattcctgtgatttaaatgtggagatgg
7.1 14T32_int_mut6  cagaaagaagggattcctgtgatttaaatgtggagatgg
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7.1 14T32_int_mut9  cagaaagaagggattcctgtgatttaaatgtggagatgg
7.1 14T32_int_mut10 cagaaagaagggattcctgtgatttaaatgtggagatgg
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7.2 14NAS_int_mut1  cagaaagaagggattcctgtgatttaaatgtggagatgg
7.2 14NAS_int_mut2  cagaaagaagggattcctgtgatttaaatgtggagatgg
7.2 14NAS_int_mut3  cagaaagaagggattcctgtgatttaaatgtggagatgg
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7.2 14NAS_int_mut21 cagaaagaagggattcctgtgatttaaatgtggagatgg
7.2 14NAS_int_mut22 cagaaagaagggattcctgtgatttaaatgtggagatgg
Fig S2
A

Donor sites

Osm5NA    TTGTGAGGTCTGTGTTA ... GAGTTAACCCTGTTTAG
          TTGTGAGGTCTGTGTTA ... CCCTCCAGTTAACCGTTTAG
          TTGTGAGGTCTGTGTTA ... CCCTCCAGTTAACCGTTTAG
          TTGTGAGGTCTGTGTTA ... CCCTCCAGTTAACCGTTTAG
          TTGTGAGGTCTGTGTTA ... CCCTCCAGTTAACCGTTTAG
          TTGTGAGGTCTGTGTTA ... CCCTCCAGTTAACCGTTTAG

Osm17NA   TTGTGAGGTCTGTGTTA ... GAGTTAACCCTGTTTAG
          TTGTGAGGTCTGTGTTA ... AGTTAACCCTGTTTAG
          TTGTGAGGTCTGTGTTA ... GAGTTAACCCTGTTTAG
          TTGTGAGGTCTGTGTTA ... GAGTTAACCCTGTTTAG
          TTGTGAGGTCTGTGTTA ... GAGTTAACCCTGTTTAG
          TTGTGAGGTCTGTGTTA ... GAGTTAACCCTGTTTAG

Osm1NA    TTGTGAGGTCTGTGTTA ... AACCCTGTTTAG
          TTGTGAGGTCTGTGTTA ... AACCCTGTTTAG
          TTGTGAGGTCTGTGTTA ... AACCCTGTTTAG
          TTGTGAGGTCTGTGTTA ... AACCCTGTTTAG
          TTGTGAGGTCTGTGTTA ... AACCCTGTTTAG
          TTGTGAGGTCTGTGTTA ... AACCCTGTTTAG

Osm9NA    TTGTGAGGTCTGTGTTA ... AACCCTGTTTAG
          TTGTGAGGTCTGTGTTA ... AACCCTGTTTAG
          TTGTGAGGTCTGTGTTA ... AACCCTGTTTAG
          TTGTGAGGTCTGTGTTA ... AACCCTGTTTAG
          TTGTGAGGTCTGTGTTA ... AACCCTGTTTAG
          TTGTGAGGTCTGTGTTA ... AACCCTGTTTAG

Osm19NA   TTGTGAGGTCTGTGTTA ... AACCCTGTTTAG
          TTGTGAGGTCTGTGTTA ... AACCCTGTTTAG
          TTGTGAGGTCTGTGTTA ... AACCCTGTTTAG
          TTGTGAGGTCTGTGTTA ... AACCCTGTTTAG
          TTGTGAGGTCTGTGTTA ... AACCCTGTTTAG
          TTGTGAGGTCTGTGTTA ... AACCCTGTTTAG

Ost8      TTGTGAGGTCTGTGTTA ... AACCCTGTTTAG
          TTGTGAGGTCTGTGTTA ... AACCCTGTTTAG
          TTGTGAGGTCTGTGTTA ... AACCCTGTTTAG
          TTGTGAGGTCTGTGTTA ... AACCCTGTTTAG
          TTGTGAGGTCTGTGTTA ... AACCCTGTTTAG
          TTGTGAGGTCTGTGTTA ... AACCCTGTTTAG

Ost16     TTGTGAGGTCTGTGTTA ... AACCCTGTTTAG
          TTGTGAGGTCTGTGTTA ... AACCCTGTTTAG
          TTGTGAGGTCTGTGTTA ... AACCCTGTTTAG
          TTGTGAGGTCTGTGTTA ... AACCCTGTTTAG
          TTGTGAGGTCTGTGTTA ... AACCCTGTTTAG
          TTGTGAGGTCTGTGTTA ... AACCCTGTTTAG

B

Insertion sites

Ost35     AAACCGTTA-Ost35---TAATCGAC
Osm5NA    AGTTATGTA-Osm5NA-TATATTTCA
          AAATAAATA-Osm5NA-TACTATTTCA
          TCTTTATGTA-Osm5NA-TATGAAAT
          TAAATACCTA-Osm5NA-TAAGAGTA

Fig S3
Fig S4
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![Image of gel electrophoresis](image)

Fig S5