Letter to the Editor

Phylogenetic Perspective Reveals Abundant Ty1/Ty2 Hybrid Elements in the *Saccharomyces cerevisiae* Genome

I. King Jordan[1] *and John F. McDonald*

Department of Genetics, University of Georgia

Retrotransposons are a class of repetitive mobile elements which transpose via the reverse transcription of an RNA intermediate (Boeke et al. 1985). These eukaryotic elements are abundant and widespread and are hypothesized to be of major evolutionary significance (Miller, Kruckenhauser, and Pinsker 1996; Kidwell and Lisch 1997; McDonald 1998). The yeast Ty retrotransposons (Ty1–Ty5) are arguably the best-characterized retrotransposons (Boeke 1989). A vast number of studies have elucidated in detail the mechanisms of Ty retrotransposition and the molecular interactions between Ty elements and their host genomes (Garfinkel 1992). The sequencing of the *Saccharomyces cerevisiae* genome (Goffeau et al. 1996) provides an unprecedented opportunity to examine the patterns of molecular variation existing among an entire complement of retrotransposons residing within a genome. Detailed analysis of these Ty element sequences promises to yield deep insight into the nature of Ty element evolution and retroelement evolution in general. Recent studies demonstrate the potential power of such analyses and have shed new light on retroelement–host coevolution (Hani and Feldman 1998; Jordan and McDonald 1998; Kim et al. 1998).

We recently performed phylogenetic analyses on sequence alignments of Ty1 and Ty2 elements characterized during the yeast genome project. Also included in our analyses were previously reported Ty1 (Ty1-H3 and Ty1-912) and Ty2 (Ty2-117) sequences (Clare and Farabaugh 1985; Warmington et al. 1985; Boeke et al. 1988). The Ty1 and Ty2 element families consist of closely related elements that are similar in size and sequence. These elements consist of two long terminal repeats (LTRs), known as δ sequences, which flank two open reading frames (ORFs) *TYA* and *TYB*. The δ sequences are made up of the U3-R-U5 regions as defined by the initiation and termination of transcription (Boeke et al. 1985). Ty1 and Ty2 elements were previously thought to share similar δ sequences but differ in their open reading frames (Curcio and Garfinkel 1994). The *TYA* ORF is homologous to the *gag* locus of retroviruses and encodes structural proteins of the viral-like particle (Clare and Farabaugh 1985). *TYB* is homologous to the *pol* locus and encodes the catalytic proteins protease (PR), integrase (IN), reverse transcriptase (RT), and RNAse H (RH) (Clare and Farabaugh 1985).

Previously reported Ty1 and Ty2 sequences, as well as those obtained from the *S. cerevisiae* Genome Database (http://genome-www.stanford.edu/Saccharomyces/), the genomic location of which can be found at the Daniel Voytas lab homepage (http://www.public.iastate.edu/~voytas/ltrstuff/ltrtables/yeast.html), were aligned using the PILEUP program of the Wisconsin GCG computer package. We performed independent phylogenetic reconstructions on seven different genomic regions of the Ty sequences (fig. 1*A*) using the neighbor-joining (Saitou and Nei 1987) option of the PHYLIP program (Felsenstein 1991). Each resulting tree consists of two major clades, the Ty1 clade and the Ty2 clade, which are separated by a long internal branch and supported with 100% bootstrap values. The U3 and RH regions of a number of Ty elements previously designated ''Ty1'' (fig. 1*A*, Ty1/2) group in the Ty2 clade separate from the Ty1 sequences. This includes 14 of the 32 elements characterized in the genome project designated ''Ty1'' and both previously reported ''Ty1'' sequences (Ty1-H3 and Ty1-912) analyzed here. Our results indicate that these so-called ''Ty1'' elements are actually Ty1/Ty2 hybrid elements. Close examination of the distribution of phylogenetically informative sites in the Ty1/Ty2 sequences allowed us to determine the recombinant breakpoints in these hybrid elements (Maynard Smith 1992) (fig. 1*B*). The locations of these breakpoints indicate that the recombination events which generated the hybrids likely occurred due to two RT-mediated template switches (fig. 2*A* and *B*) (Jordan and McDonald 1998).

Until this time, Ty1/Ty2 hybrids have not been recognized as a component of the endogenous Ty population (Curcio and Garfinkel 1994). Furthermore, Ty1/Ty2 hybrids were rarely found via selection for recombinants or insertions (Kupiec and Petes 1988; Wilke et al. 1989). This has led to the conclusion that the formation and/or maintenance of Ty1/Ty2 hybrids is defective (Curcio and Garfinkel 1994). A recent genomewide survey of *S. cerevisiae* Ty element sequences failed to detect the presence of Ty1/Ty2 hybrid sequences (Kim et al. 1998; Sandmeyer 1998). The apparent absence of recombination between Ty1 and Ty2 elements led the authors of this paper to suggest that recombination between the two families may be suppressed (Kim et al. 1998). However, our finding that nearly half of the *S. cerevisiae* elements initially identified as ''Ty1'' are actually Ty1/Ty2 hybrids suggests that recombination between the two families has occurred and that the resulting hybrid elements are in fact active and viable. Nothing about the sequences of these hybrids suggests that they are dead elements. Moreover, our revelation that previously characterized ''Ty1'' sequences such as Ty1-H3, which has been used

[1] Present address: Department of Biological Sciences, University of Nevada, Las Vegas, Nevada.

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Address for correspondence and reprints: I. King Jordan, Department of Biological Sciences, University of Nevada, 4505 Maryland Parkway, Box 454004, Las Vegas, Nevada 89154-4004. E-mail: king@parvati.lv-whi.nevada.edu.

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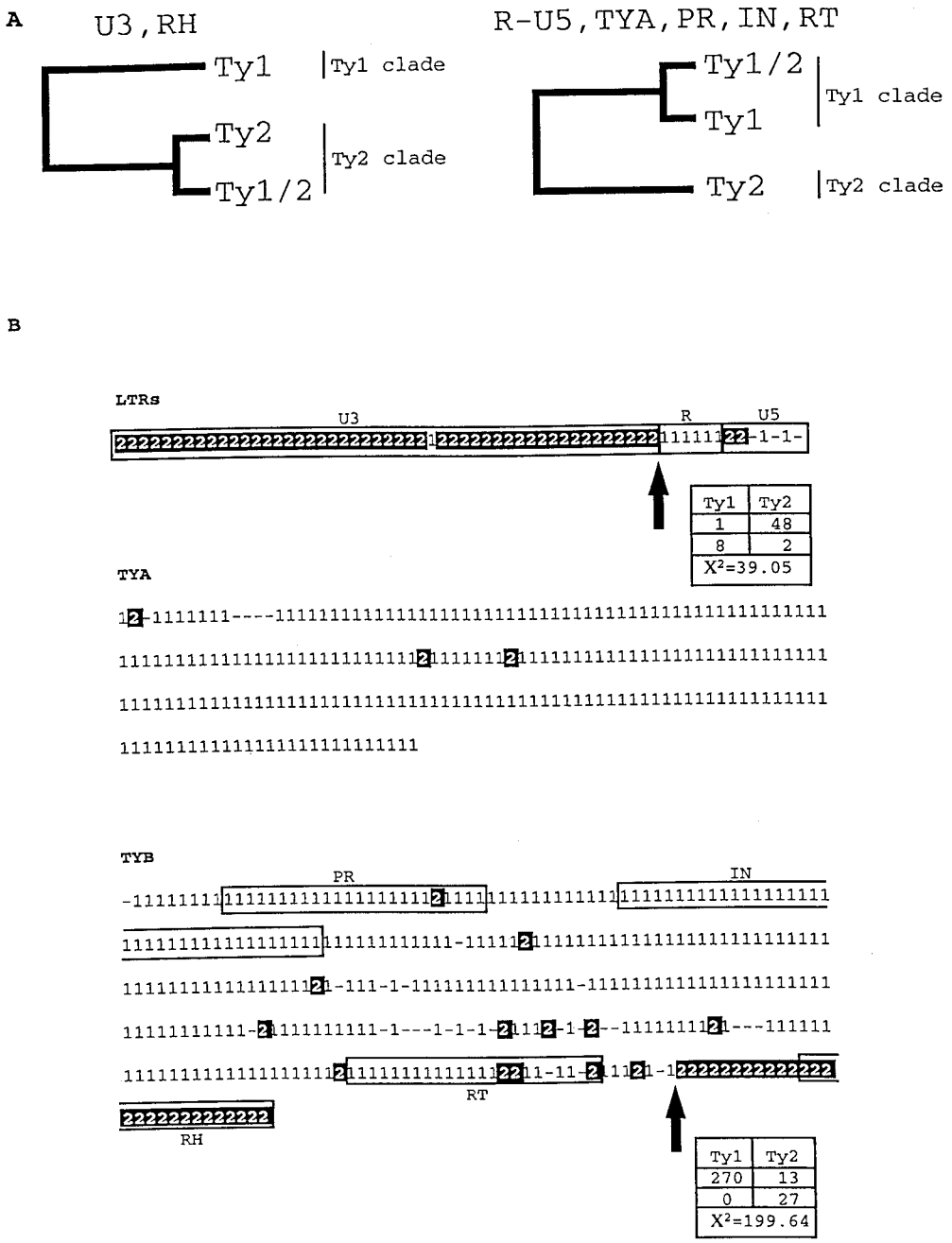

FIG. 1.—*A,* Summaries of phylogenetic reconstructions of the sequence alignments corresponding to seven regions of the Ty1 and Ty2 genomes (see text for abbreviations). Trees were constructed using DNA sequences for the LTRs and amino acid sequences for the ORFs with the neighbor-joining option of PHYLIP. Each of the two major clades in all trees were supported with 100% bootstrap values (100 replicates). *B,* Distributions of variable sites in a Ty1/Ty2 hybrid element, relative to parental Ty1 and Ty2 sequences, are shown for the different regions of the element. Phylogenetically informative sites which group the hybrid element with Ty1 elements are indicated with a 1, and sites which group the hybrid element with Ty2 are indicated with a highlighted 2. Recombinant breakpoints in the hybrid sequence are indicated with an arrow and were determined by maximizing the 2×2 χ^2 value corresponding to the distribution of Ty1 versus Ty2 phylogenetically informative sites in the hybrid sequence before and after the breakpoint.

in a number of assays for Ty1 activity (Boeke et al. 1985), are actually Ty1/Ty2 hybrids indicates that such hybrid sequences are capable of transposition and that hybrid RT-RH complexes produced from these elements are capable of acting in *trans* on other sequences.

Our results indicate that the *S. cerevisiae* genome contains numerous active Ty1/Ty2 hybrid elements in addition to parental Ty1 and Ty2 elements. Parental Ty1 and Ty2 sequences are unique in both the LTRs and the ORFs of the elements. The previous designation of all Ty1 and Ty2 LTR sequences as δ sequences was a result of the confusion of Ty1/Ty2 hybrid elements with Ty1 elements. For this reason, we propose a new designation, φ, for unique parental Ty2 LTR sequences.

Our findings raise additional questions concerning the nature of the regulatory and transpositional proper-

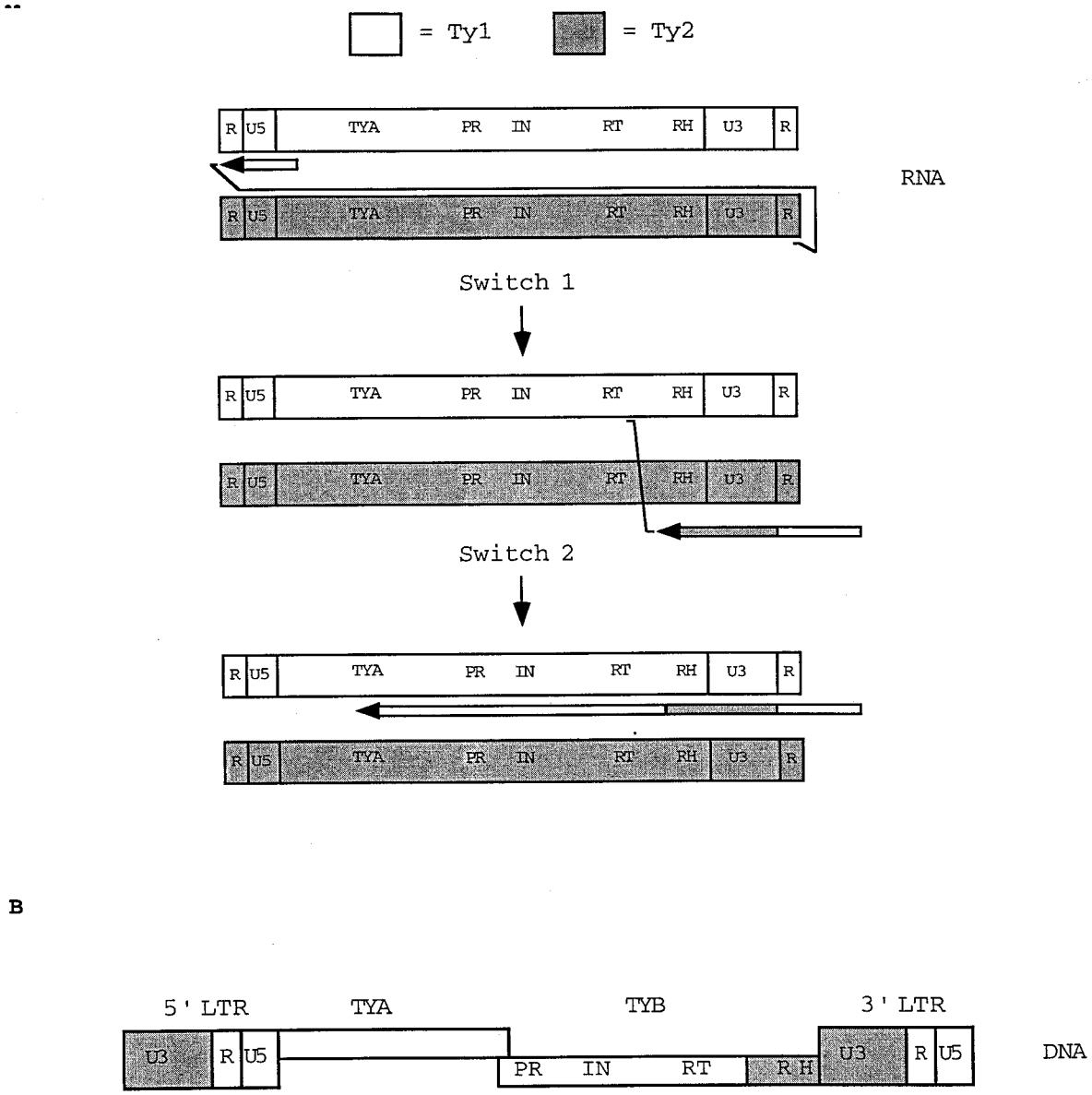

FIG. 2.—*A,* Template switching of the nascent DNA transcript between heterologous Ty1 and Ty2 RNA transcripts during the reverse transcription process. *B,* Hybrid Ty1/2 DNA provirus which results from reverse-transcription-mediated template switching.

ties of parental versus hybrid retroelements. The activity of the hybrid Ty1/Ty2 lineage is likely due to a novel regulatory phenotype resulting from the combination of Ty1 and Ty2 regulatory sequences as well as the hybrid Ty1-RT/Ty2-RH complex. The fitness of the hybrid lineage suggests that recombination may be an effective mechanism for the generation of new active classes of retroelements. It is currently not known how general a phenomenon this may be among retroelement families. LINE-like elements are thought to acquire promoter sequences via recombination (Adey et al. 1994), and recombination is prevalent among retroviruses (McClure 1996). Further analysis of endogenous retroelement variation, made possible by genome-sequencing projects, will help reveal the extent to which recombination has shaped element populations.

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