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Ty1 insertions in intergenic regions of the genome of *Saccharomyces cerevisiae* transcribed by RNA polymerase III have no detectable selective effect

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Abstract

The retrotransposon Ty1 of *Saccharomyces cerevisiae* inserts preferentially into intergenic regions in the vicinity of RNA polymerase III-transcribed genes. It has been suggested that this preference has evolved to minimize the deleterious effects of element transposition on the host genome, and thus to favor their evolutionary survival. This presupposes that such insertions have no selective effect. However, there has been no direct test of this hypothesis. Here we construct a series of strains containing single Ty1 insertions in the vicinity of tRNA genes, or in the rDNA cluster on chromosome XII, which are otherwise isogenic to strain 337, containing zero Ty1 elements. Competition experiments between 337 and the strains containing single Ty1 insertions revealed that in all cases, the Ty1 insertions have no selective effect in rich medium. These results are thus consistent with the hypothesis that the insertion site preference of Ty1 elements has evolved to minimize the deleterious effects of transposition on the host genome.

Keywords: Evolution; Continuous culture; Far3

1. Introduction

The five transposable elements which have been described in *Saccharomyces cerevisiae* belong to two families. Ty1, Ty2, Ty4 and the most recently isolated Ty5 belong to the Ty1-*copia* family whereas Ty3 is a member of the Ty3-*gypsy* group. All these elements share a common feature; they show a marked preference for transposition into intergenic regions, and regions of low transcriptional activity. In particular, Ty1, the best studied of the five elements inserts preferentially into a window of about 750 bp upstream of tRNA genes [1,2]. Thus, analysis of the complete genomic sequence of *S. cerevisiae* has shown that ~90% of all of the insertions generated by Ty1, including solo deltas, were associated with tRNA genes [3]. Nevertheless, it is well known that retrotransposons can generate a broad spectrum of mutations in their hosts, with deleterious (e.g. [4]), advantageous [5–7] and putatively neutral effects. The capacity of random insertions into the genome to disrupt genes essential for viability and growth has led to an emphasis on the deleterious effects of transposition. Accordingly Boeke and Devine [8] have suggested that the targeting of retrotransposons such as Ty1, to intergenic regions and regions of low transcriptional activity, has evolved to minimize any deleterious effects, and thus favoring their survival over evolutionary time. This argument is an extension of the idea that transposable elements can be considered to be 'selfish DNA' elements [9,10], namely that transposable elements are genomic parasites, capable of maintaining themselves in a host cell, by causing only neutral or mildly deleterious effects in that host.

However, there is some evidence suggesting that insertions in intergenic regions may indeed have a selective effect. For example, it has been shown that tRNA levels can be altered by nearby Ty insertions [11,12]. In addition, it is known that tRNAs are involved in the regulation of key steps in Ty1 transposition [13].

The selective effects of insertions in such regions have

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never been tested directly. In this communication, we show that insertions in intergenic and untranscribed regions have no detectable selective effect in laboratory culture, lending support to the conjecture that targeting of Ty1 transposition has evolved to minimize any deleterious effects.

2. Materials and methods

2.1. Media, growth and sampling

Batch cultures were grown in 10 ml 1% yeast extract, 2% peptone, and 2% glucose (YPD), at 30°C, in a gyratory shaker at 150–200 gyrations per minute. Solid YPD media contained 1.4% agar. Synthetic complete media lacking uracil (SC-ura) or leucine (SC-leu) was made as described [14], except when galactose was substituted for glucose as the carbon source for induction of pGTyH3 (see below). Cell densities were measured using an electronic particle counter (Coulter, Hialeah, FL, USA). Samples from liquid culture were stored in 40% glycerol at −70°.

2.2. Strains and strain construction

All strains were derived from strain 337, containing zero Ty1 elements [6]. To generate strains containing a single Ty1 insertion, cells were transformed with pGTyH3, a plasmid containing a transposition-competent Ty1 element under the control of the *GAL1* promoter, and the *URA3* gene as a selectable marker [15]. Colonies were picked from SC-ura plates and grown at 30° in liquid minimal media with galactose as a carbon source, to induce Ty1 transcription and transposition. Samples of cells were taken from liquid cultures at every 6–12 h. DNA was prepared from individual clones from different time points, and Ty1 number was determined by Southern analysis. From 112 clones screened in this way, clones containing only a single insertion were isolated, suspended in 40% glycerol and frozen at −70° for further analysis.

2.3. DNA manipulations and analysis

Yeast nuclear DNA was prepared using methods previously described [14]. Northern blotting experiments were carried out using methods previously described [5].

Flank polymerase chain reaction (PCR) [16] was used as follows, to isolate and clone the sequences adjacent to Ty1. One μg genomic DNA was digested with any or all of the following in individual reactions: *Eco*RV, *Nru*I, *Hpa*I, *Dra*I, *Sca*I. Sequencing adapters were ligated to restriction fragments in a 20 μl reaction including 0.5 μg digested DNA, 100 pmol flanking sequence adapter, 1.5 U T_4 DNA ligase, 1.5 mM ATP. The sequences of the flanking sequence adapter and the primers complementary to it have been described elsewhere [16]. The Ty1-specific primer 5′-GGAGTGCTCAGAGGCGTTCCAACTGATGAT-3′ and an adapter-specific primer were used for a first-round PCR amplification. A second round of amplification was performed on a 1:100 dilution of the products in the first one, using nested primers complementary to the Ty1 and the adapter. Ty1 nested flank primer has the sequence 5′-GTAAAATGACCAACCAGATGGATTGGCTTGG-3′ and is located 89 bp upstream of the first Ty1 primer. Nested products were analyzed on, and excised from a 1% agarose gel, and purified using the Qiagen gel extraction kit. In some cases products were cloned into the pCR-XL-TOPO vector available from Invitrogen (Carlsbad, CA, USA).

The cloned products were then sequenced, and their genomic location identified by comparison with the *S. cerevisiae* genome database (http://genome-www.stanford.edu/Saccharomyces/ using the BLAST algorithm [17].

2.4. Competition experiments

Competition experiments between strains carrying single Ty1 insertions and the otherwise isogenic strain 337 were carried out in serial dilution in YPD at 30°C, and replicated up to five times. All serial dilutions were initiated with equal frequencies of the two strains at a cell density of $1–2\times10^3$ cells ml^{-1}. Cells were allowed to reach stationary phase ($1–2\times10^8$ cells ml^{-1}) and were then transferred to fresh media at $1–2\times10^3$ cells ml^{-1}. At each transfer, a sample was saved in 40% glycerol for storage at −70°. Approximately every 16–33 generations, the frequency of single Ty-containing clones was assayed by PCR using primers specific to Ty1, as described previously [5]. The presence of a band of the correct size indicated a colony of cells containing the element. Selective coefficients from individual competition experiments were calculated from the regression of $\log(p/1-p)$ against time measured in generations, where p is the frequency of one of the two strains in competition. Standard population genetic theory (e.g. [18]) predicts that the relationship between $\log(p/1-p)$ and time should be linear, providing that selective effects are not dependent on frequency. The slope of the regression line estimates the selective coefficient, s, which is defined as the proportional reduction in fitness. Thus, by convention, the relative fitness of two strains A and a is 1 and $1-s$, respectively, where s is the selective coefficient.

3. Results

Table 1 and Fig. 1 show the locations of seven single Ty1 insertions in the isolated strains. Evidence from other work [6] indicated that single Ty1 insertions into the genome generated in this fashion, maintain their transpositional competence. Of these seven, six were located upstream of tRNA genes, over a range of distances, from

Table 1
Characteristics of Ty1 insertions

Clone	Chr location	Ty orientation	Site of insertion	Orientation of disrupted sequence	Nearest tRNA	tRNA orientation	tRNA sequence location	Distance to nearest tRNA (bp)	Nearest ORF	Distance to closest ORF (bp)	ORF orientation
VB20	XV 854 529[a]	w[b]	Sigma	w	Ala	c	854 254	347	SNF2 855 142	613	c
VB30	XII 459 933[c]	w	rDNA cluster	c	NA[b]	–	–	–	–	–	
VB40	X 415 778	c[b]	Between Tau and tRNA	c	Trp	c	415 724	54	YJL010c 417 250	1472	c
VB50	XI 458 069	w	Delta	c	Leu	w	458 195	126	*FOX2* 456 692	1377	c
VB70	X 396 594[a]	c	Tau	c	Gly	c	396 492	102	*PET130* 397 051	457	c
VB300	V 138 472[a]	w	Delta	w	Arg	c	138 666	194	YEL010w 136 629	1843	w
337-4	XV 438 429	w	Delta	w	Lys	w	438 644	215	ASE1	2083	c

[a]Position of insertion identified from sequencing from the plasmid, not the Ty1 element.
[b]w and c symbolize 'watson' and 'crick' strands, respectively. The watson strand is represented as the 'top' of the DNA double helix, with its transcriptional orientation 5′ to 3′ going from left to right. The crick strand is the 'bottom' and in the reverse orientation. NA: not applicable.
[c]Insertion localized to the 3′ region of the 5S RNA gene. There are over 100 clusters of rDNA in the yeast genome. This represents one of them.

54 to 215 bp away from the genes. Proximity to protein coding genes was variable; the closest insertion was located 457 bp away from *PET130* (VB70), while the farthest was over 2.0 kb away from *ASE1*. These distances were calculated as the proximity to the nearest coding sequence in either orientation, without an intervening sequence element, excluding LTRs. In only two strains, VB50 and 337-4, were insertions found upstream of protein coding genes in an orientation where they could potentially affect gene regulation. However, in both cases the loci of Ty1 integration were greater than 1.0 kb away from the gene, and so are unlikely to affect the expression of the genes. The seventh strain, VB30 possessed a single Ty1 insertion into the rDNA cluster on chromosome XII, 136 bp downstream of the 5S RNA gene (*RDN5*). The 5S RNA gene is transcribed by RNA polymerase III and has been shown previously to be an effective target for Ty1 insertion [19]. The rDNA cluster of genes has been shown to silence transcription of some RNA polymerase II-transcribed genes [19,20]. In previous work [19], it was shown that insertion of a marked Ty1 element near the 5S RNA gene results in transcriptional silencing of the element. Northern analysis of steady-state Ty1 mRNA levels showed that transcription of the single Ty1 in VB30 is similarly repressed (data not shown).

Table 2 shows the average selective coefficients for each strain. In all cases, the average selective coefficient was very small; the absolute value ranged from a low of 0.000475 to a high of 0.0134, and in every case was not significantly different from zero. By comparison, Table 2 also shows the results of a similar series of competition experiments for a Ty1 insertions upstream of the *Far3* locus [5]. In contrast the selective effect of this insertion was significantly different from zero, illustrating the power of such experiments to detect small selective effects.

Table 2
Selective coefficients

Tester strain	*N*	Selective coefficient[a]	95% CI[b]
337-4	2	−0.00378	0.0166
VB300	5	−0.00849	0.0224
VB70	2	0.00475	0.109
VB50	2	−0.012	0.0127
VB40	2	−0.0134	0.109
VB30	4	−0.000475	0.0248
VB20	4	−0.01205	0.0167
*Far3*Δ::*Kan*MX	11	−0.0204	0.0042

[a]The average selective coefficients of for each strain. Selective coefficient is calculated as the slope of the line $\ln[p/(1-p)]$ versus time in generations, where p is the relative frequency of the tester strain. Strain *Far3*Δ::*Kan*MX was constructed in X1280, and is otherwise isogenic to this strain. This strain was competed against X2180 rather than 337. See [5] for more details.
[b]95% confidence interval calculated assuming a normal distribution of the selective coefficients calculated from the individual competition experiments.

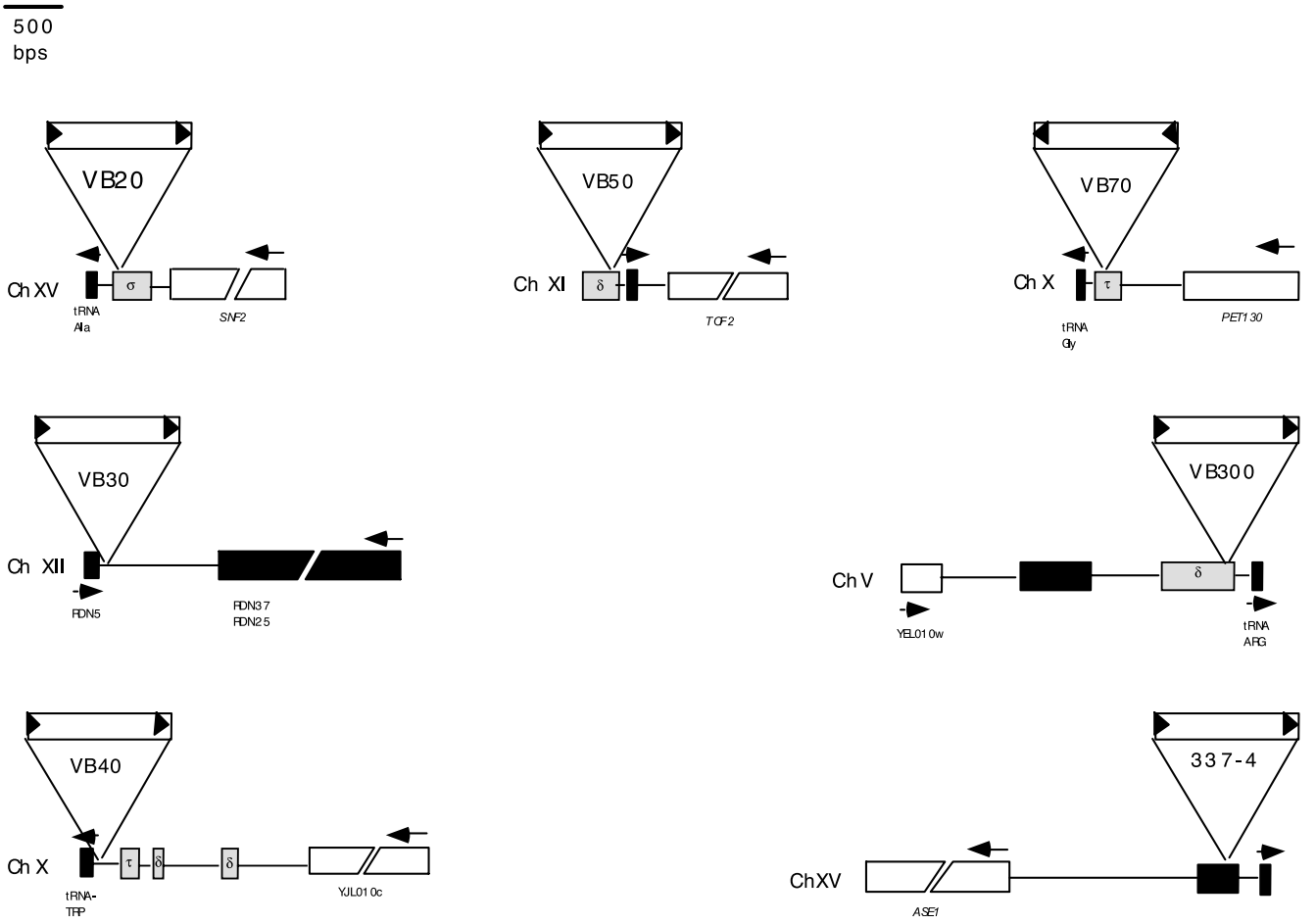

Fig. 1. Schematic representation of Ty1 insertions in the strains studied. Newly inserted Ty1 is represented by an open box with triangles at either end to denote LTRs, which also designate the orientation of transcription of the Ty1 element. Open reading frame (ORF) diagrams are based on those from the *Saccharomyces* genome database (SGD). The chromosome number is located to the left of each figure. Filled boxes are tRNA genes and stippled boxes are genomic LTRs from previous transposition events. Striped boxes represent rDNA. ORFs are open boxes and known ORFs are shown with their gene name, while unknown ORFs are described by their SGD identification. Ty1 insertions are not on the same scale as the ORF diagram. Note that in VB70, there is a τ present in the diagram. This sequence element is absent in S288c. Arrow heads show the orientation of transcription of genomic sequences. For detailed information regarding these insertions, see Table 1.

4. Discussion

The data reported here indicate that single Ty1 insertions into 5′ regions of RNA polymerase III-transcribed genes have no detectable selective effect under the conditions in which they were isolated. In the case of VB30, in which the insertion of the single Ty1 element occurred downstream of the 5S rRNA gene within the rDNA cluster on chromosome XII, it is not surprising that the Ty1 insertion shows no selective effect. Ty1 has been shown to be silenced effectively upon insertion into rDNA [19], and our results confirm that transcription of the Ty1 element in VB30 is repressed. Thus, the effect of transcriptional silencing in this strain is the strain phenotypically identical to 337.

The experimental design which we employed allowed us to detect selective effects as small as 2%. We must also consider the possibility that multiple Ty1 insertions, each one with an undetectable effect on fitness, have additive effects on fitness, such that multiple Ty1 insertions do have a detectable effect. In addition, it is possible that an environmental condition exists in which Ty1 insertions may possess a selective effect.

In conclusion, the data reported here are consistent with the hypothesis that the insertion site preference of Ty1 elements has evolved to minimize the deleterious effects of transposition on the host genome. Ty elements and *S. cerevisiae* have had a long lasting evolutionary relationship. The propensity for Ty1 to generate neutral mutations, thereby enabling its perpetuation, and its ability to generate beneficial genetic variation, suggest a host-element symbiosis.

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