Tagging chromatin with retrotransposons: target specificity of the *Saccharomyces* Ty5 retrotransposon changes with the chromosomal localization of Sir3p and Sir4p

Yunxia Zhu,¹ Sige Zou,^{1,2} David A. Wright, and Daniel F. Voytas³

Department of Zoology and Genetics, Iowa State University, Ames, Iowa 50011 USA

Retrotransposon and retroviral insertions are not randomly distributed on chromosomes, suggesting that retroelements actively select integration sites. This is the case for the yeast Ty5 retrotransposons, which preferentially integrate into domains of silent chromatin at the *HM* loci and telomeres. Here we demonstrate that loss of Sir3p or Sir4p—components of silent chromatin—causes a greater than ninefold decrease in Ty5 targeting to the *HM* loci and largely randomizes chromosomal integration patterns. Strains with a deletion of *SIR4* also display an ~10-fold increase in cDNA recombination, which is due both to the expression α - and α -mating-type information and the loss of Sir4p. It is known that in old yeast cells or in strains carrying the *sir4-42* allele, the Sir complex relocates to the rDNA. About 26% of Ty5 insertions occur within the rDNA in *sir4-42* strains compared with 3% in wild type. Ty5, therefore, is sensitive to changes in chromatin, indicating that retrotransposons may be useful for dissecting chromatin dynamics that occur during developmental programs such as aging.

[**Key Words:** Retrotransposon; integration; silent chromatin; recombination; target specificity]

Received May 24, 1999; revised version accepted August 30, 1999.

Reverse transcription as carried out by the retroviruses and retrotransposons occurs within a nucleoprotein complex in the cell cytoplasm. The resulting cDNA is transported into the nucleus as part of an integration complex, a key component of which is the retroelement-encoded integrase (Brown 1997). Integrase carries out the strand scission and joining reaction that inserts the cDNA into the host genome. The nonrandom chromosomal distribution of retrovirus and retrotransposon insertions suggests that the integration complex discriminates among potential integration sites (Sandmeyer et al. 1990; Craigie 1992). Integration specificity is particularly pronounced for the retrotransposons, which are obligate genomic parasites, and targeting may allow retrotransposons to persist by avoiding the disruption of genes or other sequences critical for host survival (Boeke and Devine 1998).

To explain retroelement integration site selection, one widely held model suggests that the retroelement inte-

gration complex interacts with DNA-bound proteins or components of chromatin (Bushman 1995). This serves to tether the integration machinery to the target site, resulting in the observed target site biases. The most compelling support for this model comes from the study of the *Saccharomyces cerevisiae* retrotransposons (five families, designated Ty1–Ty5) (Boeke and Sandmeyer 1991). In the *S. cerevisiae* genome, >90% of native Ty1–Ty4 insertions are found in the upstream regions of genes transcribed by RNA polymerase III (Pol III), such as tRNA genes (Kim et al. 1998). For Ty1 and Ty3, this genomic organization is due to targeted integration, which requires the assembly of the RNA Pol III transcription complex (Chalker and Sandmeyer 1992; Devine and Boeke 1996). Mutations that abolish the binding of Pol III transcription factors abolish targeted integration. For Ty3, the transcription factors TFIIB and TFIIC are sufficient for targeting in vitro when they are loaded onto a tRNA gene template (Kirchner et al. 1995). Thus the Ty1 and Ty3 integration complexes interact with a component of the RNA Pol III transcriptional apparatus or recognize a chromatin state associated with RNA Pol III transcription.

The Ty5 elements display a markedly different target bias—>90% of Ty5 integration events occur near the

¹These two contributed equally to this work.

²Present address: Howard Hughes Medical Institute, University of California at San Francisco, San Francisco, California 94143.

³Corresponding author.

E-MAIL voytas@iastate.edu; FAX (515) 294-6755.

telomeres or silent mating loci, *HML* and *HMR* (Zou et al. 1996a,b). The telomeres and *HM* loci are bound in silent chromatin, which is analogous to heterochromatin of higher eukaryotes (for recent reviews, see Loo and Rine 1995; Lustig 1998). Silent chromatin has a number of cellular functions—it represses transcription of the *HM* loci, is involved in telomere maintenance, and plays a role in senescence. Components of silent chromatin assemble at specific *cis*-acting sequences, which for the *HM* loci are the flanking transcriptional silencers designated E and I. These silencers bind the transcriptional regulators Rap1p and Abf1p as well as the origin recognition complex (ORC). These same proteins also bind to sequences near the telomeres, either to the telomeric TG₁₋₃ repeats (Rap1p) or to sites within the subtelomeric X repeats (Abf1p and ORC). These DNA-binding proteins nucleate the assembly of the many other components of silent chromatin, including the well-studied silent information regulators, Sir1p–Sir4p. Sir1p acts primarily to establish silencing at the *HM* loci, whereas Sir2p, Sir3p, and Sir4p are considered structural components of silent chromatin. They nucleate at the silencers and spread out to create a hypoacetylated chromatin domain.

Studies of the requirements for Ty5 target specificity support the general model that integration complexes recognize chromatin or other DNA-bound proteins such as transcription factors. Ty5 typically integrates within an ~3-kb window encompassing the *HM* transcriptional silencers or the subtelomeric X repeat (Zou et al. 1996a, b). Silent chromatin determines Ty5 target preference, because mutations in *HMR-E* that fail to assemble silent chromatin also fail to direct Ty5 integration (Zou and Voytas 1997). Mutations in individual protein-binding sites within the silencer, however, have only modest effects on targeting, suggesting that the integration complex may not interact directly with DNA-bound proteins (e.g., Rap1, Abf1, ORC), but rather with the factors they recruit to establish the silent state. Studies of Ty5-encoded factors required for targeting suggest that integrase itself may physically interact with a component of silent chromatin to mediate target choice. A single amino acid change near the carboxyl terminus of Ty5 integrase was found to randomize Ty5 integration patterns (Gai and Voytas 1998).

Chromatin states are not static. Reorganization of chromatin, which occurs during the onset of particular developmental programs, may alter retroelement targeting patterns. In *S. cerevisiae*, the rDNA is another domain of silent chromatin, and rDNA silencing depends on the silent information regulator Sir2p (Bryk et al. 1997; Smith and Boeke 1997). Sir3p and Sir4p dramatically change their distribution between the telomeres/*HM* loci and the rDNA as yeast cells age. The first evidence for this was provided by the isolation of a carboxy-terminal truncated allele of *SIR4* (*sir4-42*), which prolongs the life span of yeast cells (Kennedy et al. 1995). It was proposed that *sir4-42* mediates its effect by preventing recruitment of the Sir complex to the telomeres and *HM* loci, thereby increasing their concentration at

hypothetical *AGE* loci—regulators of life span. Indirect immunofluorescence studies showed that in both *sir4-42* and old wild-type cells, Sir3p and Sir4p relocate from the telomeres to the rDNA (nucleolus), suggesting that the rDNA is the *AGE* locus (Kennedy et al. 1997). More recently, rDNA breakdown has been shown to mark the onset of senescence in yeast, and yeast life span is measured by a clock calibrated to the accumulation of rDNA circles that result from recombination between rDNA repeats (Sinclair et al. 1998). The relocation of the Sir complex to the rDNA during aging or in *sir4-42* strains likely reorganizes chromatin and mitigates the onset of rDNA recombination and senescence.

In this work, we further explore the model that retroelement target specificity results from the recognition of specific chromatin components during integration. In particular, we analyze Ty5 integration patterns in strains with deletions of individual *SIR* genes, and we demonstrate that in strains lacking Sir3p or Sir4p, Ty5 target specificity is largely abolished. In addition, we found that in *sir4-42* strains, wherein the Sir complex relocates from the telomeres to the nucleolus, Ty5 integrates preferentially into the rDNA. This indicates that retroelements like Ty5 are highly sensitive to changes in chromatin structure and can be used to map chromatin domains or to tag chromatin states that change during specific developmental programs.

Results

Assays for Ty5 cDNA integration and recombination

Because of the Ty5's preference to integrate into domains of silent chromatin, the Sir proteins were viewed as likely candidates for being recognized by the Ty5 integration complex. We used strains with deletions in individual *SIR* genes to assess their role in integration and recombination. In our transposition assay, Ty5 transcription is controlled by a galactose-inducible promoter, and the donor Ty5 element carries a marker gene (*his3AI*) that is rendered nonfunctional because of the presence of an artificial intron (Zou et al. 1996a; Fig. 1). When cells with the donor element are grown on galactose, transcription is induced, and the artificial intron is spliced from the Ty5 mRNA. Reverse transcription of the spliced message reconstitutes a functional *HIS3* gene. A His⁺ phenotype can result either when Ty5 cDNA integrates into the genome, recombines with the *his3AI* marker on the donor plasmid, or recombines with a chromosomal substrate (Ke and Voytas 1997, 1999). Plasmid and chromosomal events are distinguished by growing cells on synthetic complete medium without histidine and with 5-fluoroorotic acid (SC-H + 5-FOA). 5-FOA selects against the plasmid-borne *URA3* gene, and, therefore, cells in which the *HIS3* marker has recombined with the plasmid will not grow on SC-H + 5-FOA medium. Cells that survive this selection (i.e., His⁺/5-FOA^r cells) include Ty5 chromosomal integration and recombination events—the latter are typically gene conversions of the *his3-11,15* allele (see data below). These can

Figure 1. An assay to monitor Ty5 integration and recombination. The Ty5 donor element carries a *his3AI* marker gene that is interrupted by an artificial intron. The intron is in the incorrect orientation to be spliced from the *HIS3* transcript; however, it can be spliced from the Ty5 mRNA, and a functional *HIS3* gene is generated only when the spliced transcript is reverse transcribed. Ty5 cDNA can either integrate into its target or recombine with homologous substrates. Because the yeast genome contains only a few degenerate Ty5 elements (Kim et al. 1998), recombination primarily occurs with the plasmid-borne *his3AI* marker or the chromosomal *his3-11,15* allele (Ke and Voytas 1999). Plasmid recombination and chromosomal events (either recombination or integration) can be distinguished by growth on synthetic complete medium without histidine and with 5-FOA (SC-H + 5-FOA). 5-FOA selects against the plasmid-borne *URA3* gene (Boeke et al. 1987), and strains that are His⁺ because of plasmid recombination will not grow on SC-H + 5-FOA medium. Chromosomal recombination and integration are then distinguished by Southern blot analysis or PCR screens (see Materials and Methods).

be distinguished by Southern blot or PCR analysis (see Materials and Methods for details).

The effect of Sir proteins on Ty5 cDNA integration and recombination

The frequencies of His⁺ cell formation were higher in the *SIR* deletion strains than in the wild-type strain (Table

1). This was primarily due to an increase in cDNA recombination, because integration frequencies were modestly lower. The high-efficiency recombination phenotype was most pronounced for $\Delta sir4$ strains, which exhibited a >10-fold increase in the frequency of His⁺ cell formation, a very high percentage of plasmid recombination (91% vs. 38% in wild type) and a very low percentage of chromosomal integration (3.3% vs. 58.9% in wild type). Loss of the Sir proteins results in derepression of the *HM* loci and the expression of both *a*- and α -mating type information. To distinguish between contributions made directly by loss of Sir proteins as opposed to the consequential derepression of the silent mating-type loci, the *HMR* locus was deleted in each of the *MAT α* Δsir strains. In $\Delta sir1 \Delta hmr$, $\Delta sir2 \Delta hmr$, and $\Delta sir3 \Delta hmr$, the overall His⁺ frequency and the frequency of plasmid recombination were comparable with wild-type and lower than the Δsir strains, and the percentage of plasmid recombination was intermediary to the wild-type and the Δsir strains (Fig. 2). This suggests that the derepression of the silent mating loci is the primary contributor to the high efficiency of recombination and that the loss of the Sir proteins, Sir2p, and Sir3p, only contribute to the increase of the percentage recombination. A control strain (*MAT α* *HMR-E aeb*) with a silencer mutation that expresses both *a*- and α -mating-type information confirmed the contribution made by derepression of the silent mating type loci to the high recombination phenotype. For the $\Delta sir4 \Delta hmr$ strain, the frequency of His⁺ cell formation was more than four fold greater than wild type, and the percent cDNA recombination was higher than the wild-type and other $\Delta sir \Delta hmr$ strains (71.2% vs. 28.0%–53.8%). In contrast to the other double mutants, the high efficiency and high percentage of recombination phenotype appears to be the result of a synergistic effect resulting from the derepression of the silent mating-type loci and the loss of Sir4p.

An example of Southern blot analysis used to distinguish chromosomal integration and gene conversion is shown in Figure 3. Filters containing DNA from 12 independent His⁺/5-FOA^r wild-type or $\Delta sir4$ strains were hybridized with Ty5 or *HIS3* probes. In the case of the wild-type strain, 11/12 have Ty5 sequences and two copies of *HIS3*—the endogenous *his3-11,15* allele and the *HIS3* associated with Ty5. For $\Delta sir4$, only 4/12 His⁺/5-FOA^r strains carry Ty5-*HIS3*. One of the $\Delta sir4$ strains (see Fig. 3B,D) carries several copies of Ty5, which we often observe when Ty5 integration is blocked (Ke and

Table 1. Ty5 integration and recombination in wild-type and Δsir strains

Genotype	Frequency of His ⁺ cell formation (10 ⁻⁶)	Percent plasmid recombination	Percent chromosomal recombination	Percent chromosomal integration	Integration frequency (10 ⁻⁶)	Fold reduction in integration frequency
Wild type	4.64 ± 1.62	38.0	3.1	58.9	2.73	N.A.
$\Delta sir1$	5.78 ± 0.40	64.8	1.2	34.0	1.96	1.4
$\Delta sir2$	11.7 ± 0.14	87.2	2.1	10.7	1.25	2.2
$\Delta sir3$	7.56 ± 1.25	78.2	2.6	19.2	1.45	1.9
$\Delta sir4$	51.8 ± 1.77	91.0	5.7	3.3	1.71	1.6

[N.A.] Not applicable.

Figure 2. Ty5 cDNA plasmid recombination in Δsir and $\Delta sir \Delta hmr$ mutants. The frequency of His⁺ cell formation and the percentage of plasmid recombination were measured as described in the Materials and Methods and the legend to Fig. 1. All strains are MAT α . The value above each bar is the percentage of plasmid recombination.

Voytas 1999). For both the wild-type and $\Delta sir4$ strains, the presence of Ty5 sequences was scored as integration and the lack of Ty5 sequences was scored as gene conversion of *his3-11,15*.

Because of the high rates of cDNA recombination, we felt that we may have underestimated the effects of *SIR* deletions on integration. For example, it is difficult to

distinguish integration events from recombination of Ty5 cDNA with the native Ty5 elements on the yeast chromosomes. Native elements are degenerate, and although we have never previously observed such recombination events in wild-type strains, their frequency could be increased if integration were affected by *SIR* deletions. To get a more accurate estimate of the effect of *SIR* deletions on Ty5 integration, we also measured integration in $\Delta sir2$, $\Delta sir3$, and $\Delta sir4$ strains in which the *RAD52* gene was deleted to eliminate homologous recombination (Petes et al. 1991; Table 2). We found that the deletion of *RAD52* did not significantly affect integration frequency in wild-type strains. However, integration in $\Delta sir \Delta rad52$ double mutants was reduced relative to the individual Δsir strains and the $\Delta rad52$ single mutant, suggesting that recombination with the native Ty5 sequences may occur at appreciable levels in Δsir backgrounds. Alternatively, there could be a synergistic transposition defect caused by loss of the *SIR* and *RAD52* gene products; this remains to be tested.

Deletion of *SIR3* or *SIR4* impairs targeting of Ty5 to the HM loci

To determine whether the Sir proteins are important for Ty5 target specificity, we monitored integration near each of the four *HM* silencers in either wild-type strains or strains with deletion in each of four *SIR* genes. For this study, we modified a PCR assay used previously to measure targeting of Ty5 to the *HMR-E* transcriptional silencer (Zou and Voytas 1997). Transposition was induced and genomic DNA was prepared in pools representing 10 independent His⁺/5-FOA^r strains. DNA pools were PCR-amplified four times; each reaction contained two primers specific for the Ty5 long terminal repeat (LTR) and two primers flanking a given silencer (Fig. 4). The position of the primers made it possible to detect integration events within ~2-kb windows flanking each silencer, which in total represent ~0.06% of the genome. Because the His⁺/5-FOA^r strains include both chromosomal integration and chromosomal recombination events, the effective number of integration events was adjusted with the data from Table 1.

Targeting to the *HM* loci was differentially affected by the *SIR* mutations (Table 3). Deletion of *SIR1* slightly reduced targeting, whereas deletion of *SIR2* decreased

Figure 3. Gene conversion of *his3-11,15* by Ty5 cDNA is enhanced in $\Delta sir4$ strains. Ty5 transposition was induced in wild-type or $\Delta sir4$ strains, and DNA was prepared from 12 independent His⁺/5-FOA^r strains. DNA was digested with *HpaI*, which cuts 2.4 kb from the 5' end of the 6.4-kb Ty5 element. Southern filters were prepared and hybridized with an LTR probe (A,B). Filters were stripped and rehybridized with a *HIS3* probe (C,D). The presence of Ty5 in a given strain is indicated by hybridization to the LTR probe. In all cases, strains with Ty5 LTRs also had two restriction fragments that hybridized to *HIS3*—one representing the *HIS3* marker in Ty5 and the other the *his3-11,15* allele (arrow). (*) His⁺ strains lacking Ty5 sequences were considered to have arisen by gene conversion of the *his3-11,15* allele by Ty5 cDNA.

Table 2. Ty5 integration in homologous recombination-deficient yeast strains

Genotype	Integration frequency (10 ⁻⁶)	Fold reduction in integration frequency
$\Delta rad52$	2.08 ± 0.27	N.A.
$\Delta sir2 \Delta rad52$	0.30 ± 0.08	6.81
$\Delta sir3 \Delta rad52$	0.44 ± 0.11	4.68
$\Delta sir4 \Delta rad52$	0.38 ± 0.16	5.42
<i>sir4-42</i> $\Delta rad52$	0.62 ± 0.08	3.34

(N.A.) Not available.

Figure 4. A PCR assay to identify Ty5 insertions near the *HM* loci. At both the *HML* and *HMR* loci, E and I represent the flanking transcriptional silencers. W, X, and Z are homologous regions shared between the *HML*, *HMR*, or *MAT*. $Y\alpha$ and $Y\alpha$ are specific for *HML* and *HMR*, respectively. Transcripts [$\alpha 1$, $\alpha 2$] at each locus are shown by the longer horizontal arrows. Primers used in the PCR amplifications are labeled with their DVO designator. DNA was prepared in pools, each consisting of 10 independent $\text{His}^+/\text{5-FOA}^r$ strains. The pooled DNA was amplified four times to detect integration events within an ~ 2 -kb window near each of the four *HM* silencers. Each reaction included two Ty5-specific primers (DVO496, DVO495) and two primers flanking a given silencer (e.g., DVO247 and DVO212 for *HML-E*). Because most strains did not carry a Ty5 insertion within the tested interval, amplification from the primers flanking the silencer generated a fragment of ~ 2 kb; this fragment served as an internal control for the PCR reactions. Fragments < 2 kb indicated the presence of a Ty5 insertion, which was then confirmed by amplifying the pooled DNA through additional rounds of PCR with individual Ty5 and silencer primers.

targeting 3.7-fold. Targeting was reduced 9.4-fold in Δsir3 , and no targeted events were detected in the Δsir4 strain. Despite the targeting decrease in Δsir2 and Δsir3 , the frequencies of *HM* insertions were at least 10 times greater than predicted for random integration in the genome. This suggests that although targeting is impaired, the Ty5 integration complex can still preferentially recognize these loci.

Chromosomal integration patterns in Δsir strains

To determine the effect of *SIR* deletions on chromosomal integration patterns, independent transposition events in Δsir2 , Δsir3 , and Δsir4 strains were characterized. Sequences flanking Ty5 integration sites were amplified by inverse PCR, sequenced, and mapped onto the complete *S. cerevisiae* genome sequence. We typically use the ORC-binding site—the autonomously replicating consensus sequence (ACS) within either the telomeric X repeat or the *HM* silencers—as a guidepost to orient integration events. Insertions were considered targeted if they occurred within 1.5 kb of such an ACS. By this definition, $\sim 90\%$ of Ty5 transposition events are targeted in wild-type strains (Zou et al. 1996a,b).

We observed that the absence of Sir proteins altered chromosomal integration patterns (Table 4). Among ten insertions characterized in the Δsir2 strain, four met our definition of targeted events, one was within a telomeric Y' element duplicated at the ends of chromosomes IX and X, one was located in the rDNA array, and the other

four were randomly distributed in the genome. Of eleven insertions in the Δsir3 strain, one was targeted, three were clustered within the chromosome IX/X Y' element, and the remainder were distributed throughout the genome. Among nine insertions in the Δsir4 strain, one was targeted, two were within or near Ty1 elements, and the rest were randomly dispersed. As with the *HM* targeting assay, deletion of *SIR3* or *SIR4* had the most significant effect on Ty5 target specificity.

Ty5 integration occurs preferentially within Ty elements and the rDNA in *sir4-42* strains

As cells age, Sir3p and Sir4p move from the telomeres and the silent mating loci to the rDNA (Kennedy et al. 1997). This relocation is also observed in strains carrying the *sir4-42* allele, which produces a carboxy-terminal truncated SIR4 protein. We monitored Ty5 integration efficiency and targeting in a *sir4-42* strain. Compared with the Δsir4 strain, frequencies of His^+ cell formation and cDNA recombination were largely indistinguishable (data not shown). Integration efficiency in a *sir4-42* Δrad52 strain was slightly higher than in a Δsir4 Δrad52 strain (Table 2). A high frequency of cDNA recombination was observed on characterization of chromosomal insertions. Of nineteen independent chromosomal events analyzed in *sir4-42*, four were tandem elements (data not shown), which arose through recombination with the native Ty5-1 element near the left telomere of chromosome III (Voytas and Boeke 1992). Of the fifteen remaining chromosomal events, one integrated within the telomeric Ty5-1 element (Table 5). Interestingly, four were within pre-existing Ty1 or Ty3 elements, and two were within 600 bases of such elements. Retrotransposon sequences as a whole constitute 3.1% of the genome (Kim et al. 1998), and the observation that 7/15 insertions were associated with Ty elements suggests that Ty5 may be preferentially targeted to retrotransposons in *sir4-42* strains.

Three of the fifteen insertions in the *sir4-42* strain occurred within the rDNA array, which accounts for $\sim 10\%$ of the *S. cerevisiae* genome (Cherry et al. 1997). To better characterize this apparent change in target specificity, we developed a PCR assay to quantitate and map integration events within the rDNA repeats (Fig. 5A). Ty5 insertions in the rDNA were amplified by PCR using rDNA- and Ty5 LTR-specific primers. Primers that amplify an internal portion of Ty5 served as a control for the PCR reaction and for the presence of Ty5 in the genome. Because Ty5-*HIS3* cDNA can recombine with the endogenous *his3-11,15* allele at a high frequency, we used Δrad52 strains for this assay. We observed a remarkable increase in targeting to the rDNA in the *sir4-42* strain (26%) compared with wild type (3%) (Table 6). A modest (approximately twofold) increase was also observed in the Δsir2 and Δsir4 strains.

Because the position of the primers within the rDNA is fixed, it was possible to map the approximate location of the insertions on the basis of the size of the amplification products (Fig. 5B). Two clusters of insertions were

Table 3. The targeting efficiency of Ty5 to HM loci in wild-type and Δsir strains

Genotype	Chromosomal events characterized	Effective integration events ^a	Insertions near HM silencers	Percent HM targeting	Fold reduction in HM targeting
Wild-type	200	190	12	6.32	N.A.
$\Delta sir 1$	200	193	5	2.59	2.44
$\Delta sir 2$	210	175	3	1.71	3.70
$\Delta sir 3$	340	300	2	0.67	9.43
$\Delta sir 4$	230	84	0	N.A.	N.A.

(N.A.) Not applicable.

^aEffective integration events are calculated as the product of the chromosomal events characterized and the percent chromosomal integration (from Table 1) divided by the sum of the percent chromosomal integration and the percent chromosomal recombination (from Table 1).

identified; one flanks the 5S gene and the other is near the junction of the 5.8S and 25S genes. Of the three rDNA insertions in *sir4-42* that were identified by direct cloning (Table 5), two were located near the 5S gene and one was within the 25S hotspot. Similarly, of the three wild-type rDNA insertions (Table 6), two were near the

5S and 25S hotspots, and one was within the 18S gene. In addition, there was no apparent preference to integrate on either strand of the rDNA. We conclude, therefore, that in *sir4-42*, not only is Ty5 target specificity changed, but the pattern of integration events within the rDNA locus is distinctly nonrandom.

Table 4. Chromosomal distribution of Ty5 insertions in Δsir strains

Strain	Chr. no.	Coordinate	Location ^a	Nearby chromosomal features
$\Delta sir 2$				
T3	I	~600	T	within YAL069W, ~153 bases from X repeat ACS
T4	VII	287	T	446 bases from ACS in X repeat
T17	IX or X	~8424	T	~695 bases from ACS in X repeat
T10	III	293099	T	within <i>HMR</i> , 506 bases from ACS in <i>HMR-I</i>
T8	XIV	~1000	NT	within Y', 6165 bases from core X repeat
T28	XII	455677	R	between genes for 5.8S and 18S rRNA
T4	VI	1511923	D	between <i>PAD1</i> and <i>YDR539W</i>
T18	XI	67821	D	between <i>MNN4</i> and <i>YKT9</i>
T12	XIV	91046	D	within <i>YNL288W</i>
T20	XV	891622	D	between <i>YOR306C</i> and <i>SLY41</i>
$\Delta sir 3$				
H29	III	1568	T	within Ty5-1 (YCLWTy5-1), 515 bases from ACS in X repeat
H11	IX or X	87	NT	within Y', 7632 bases from ACS in X repeat
H36	IX or X	~97	NT	within Y', ~7622 bases from ACS in X repeat
H25	IX or X	395	NT	within Y', 7324 bases from ACS in X repeat
H24	I	124455	D	between <i>FUN31</i> and <i>TPD3</i>
H31	IV	131105	D	within <i>YDL183C</i>
H1	IV	1186012	D	between <i>TRP4</i> and <i>YDR355C</i>
H12	V	260751	D	between <i>YER053C</i> and <i>GIP2</i>
H6	XV	721754	D	between <i>PET56</i> and <i>HIS3</i>
H5	XVI	105640	D	between <i>TFP3</i> and <i>YPL233W</i>
H4	XVI	396718	D	within <i>SEN54</i>
$\Delta sir 4$				
F4	XII	11487	T	between <i>YLL066C</i> and <i>YLL065W</i> , 550 bases from ACS in X repeat
F47	X	483062	Ty	within Ty1 element YJRWTy1-2
F19	XII	796771	NTy	within <i>YLR333C</i> , 127 bases from Ty1 insertion
F10	IV	309450	D	between <i>RPL13A</i> and <i>RPLA1</i>
F6	VII	~330801	D	between <i>VPS45</i> and <i>PAN2</i>
F36	XI	557166	D	between <i>YKR060W</i> and <i>KTR2</i>
F3	XII	830607	D	between <i>YLR351C</i> and <i>YLR352W</i>
F37	XIV	317917	D	between <i>PSD1</i> and <i>YNL168C</i>
F9	XV	825909	D	within <i>YOR268C</i>

^a(T) Targeted insertions at the telomeres or HM loci; (NT) telomeric insertions that do not meet our definition for targeting; (R) insertions within the rDNA; (Ty) insertions within Ty elements; (NTy) insertions within 750 bases of a Ty element; (D) dispersed insertions with no apparent target specificity.

Table 5. Chromosomal distribution of Ty5 insertions in *sir4-42*

Strain	Chr. no.	Coordinate	Location ^a	Nearby chromosomal feature
A19	I	165824	Ty	within Ty1 LTR (YARCdelta5)
A68	V	496010	Ty	within Ty1(YERCTy1-2)
A129	XIII	199915	Ty	within Ty1 (YMLWTy1-2)
A126	XVI	56341	Ty	within Ty3 LTR (YPLWsigma1)
A91	V	311723	NTy	between <i>PTP3</i> and tA(UGC)E, 547 bases from Ty1 LTR (YERWdelta11)
A17	VIII	117119	NTy	65 bases from Ty1 LTR (YHRCdelta4)
A99	XII	454867	R	within gene for 25S rRNA
A103	XII	458723	R	between genes for 18S and 5S rRNA
A20	XII	460656	R	near gene for 5S rRNA
A13	III	2685	NT	within Ty5 element (YCLWTy5-1), 1632 bases from ACS in X repeat
A89	II	161776	D	within <i>SHE1</i>
A72	II	629008	D	between <i>CDC47</i> and YBR203W
A69	VIII	506793	D	within YHR204W
A62	IX	138988	D	between <i>RPI1</i> and <i>RHO3</i>
A8	XIV	733878	D	within <i>BIO4</i>

^a(Ty) Insertions within Ty elements; (NTy) insertions within 750 bases of a Ty element; (R) insertions within the rDNA; (NT) telomeric insertions that do not meet our definition for targeted events; (D) dispersed insertions with no apparent target specificity.

Discussion

Sir3p and Sir4p are important for Ty5 target specificity

A general model to explain target specificity of the retroviruses and LTR retrotransposons invokes a physical interaction between the retroelement integration complex and chromosomal proteins (Bushman 1995). This tethers the integration complex to specific chromosomal sites, leading to the observed target site biases. This model is well supported by studies of the yeast Ty5 retrotransposons. We have identified previously a domain near the carboxyl terminus of Ty5 integrase, which when mutated, abolishes the preference of this element to integrate into silent chromatin (Gai and Voytas 1998). This suggests that integrase is the chromatin sensor. We have also shown that silent chromatin is required for Ty5 target specificity. Mutations in the *HMR-E* silencer that disrupt the assembly of silent chromatin (as measured by transcriptional silencing) also abolish targeting (Zou and Voytas 1997). *HMR-E* binds three proteins or protein complexes—Abf1p, Rap1p, and ORC—which in turn help to recruit the Sir proteins (Loo and Rine 1995; Lustig 1998). Because the Sir complex is found at both the telomeres and HM loci and is required for silencing, we felt that the Sir proteins were likely candidates for being recognized by the Ty5 integration complex.

In this study, we tested Ty5 target specificity in strains with deletions in each of the four *SIR* genes. Deletion of either *SIR3* or *SIR4* resulted in a greater than ninefold decrease in integration at the HM loci. When integration patterns were characterized at the chromosomal level, only 1/10 $\Delta sir3$ insertions and 1/9 $\Delta sir4$ insertions were targeted to silent chromatin (defined as within 1.5 kb from the ACS within an HM silencer or the telomeric X repeat). This contrasts with ~90% targeting observed in wild-type strains (Zou et al. 1996a,b). In $\Delta sir1$, targeting to the HM loci was modestly affected (down 2.44-fold).

Sir1p acts primarily at the HM loci to help establish silencing (Pillus and Rine 1989; Aparicio et al. 1991), and because Ty5 integrates efficiently at both telomeres and the HM silencers, this result was not unexpected. $\Delta sir2$ strains had an intermediary effect; HM targeting was decreased 3.77-fold and 4/10 chromosomal insertions were targeted. Sir2p is required for transcriptional silencing at telomeres, HM loci, and rDNA. The observation that targeting is partially functional in $\Delta sir2$ strains indicates that transcriptional silencing and Ty5 target specificity are genetically separable.

Although Sir3p and Sir4p are important for targeting, targeted transposition events were still recovered in $\Delta sir3$ and $\Delta sir4$ strains at frequencies approximating 10%; the target window (1.5 kb from an ACS within the HM silencers or X repeat) accounts for <1% of the genome (Cherry et al. 1997). This indicates that whereas targeting is greatly diminished in $\Delta sir3$ and $\Delta sir4$ strains, insertions into silent chromatin still occur at frequencies significantly greater than random. This suggests that Sir3p and Sir4p are not the only factors important for Ty5 target specificity. The Ty5 integration complex may interact with multiple factors, or Sir3p and Sir4p may stabilize or recruit additional factors required for optimal targeting. With our recently identified Ty5 targeting domain, we are currently using a variety of genetic and biochemical means to identify candidate interactors. It is important to note, however, that at this time we cannot exclude alternative models to explain target specificity. For example, one model suggests that there may be a silent compartment within the nucleus (Boeke and Devine 1998). In such a scenario, the Ty5-encoded targeting domain would serve as a localization signal to direct the integration complex to this subnuclear compartment. Once localized, integration would take place into the available DNA, giving rise to the observed target bias.

Figure 5. (A) A PCR assay to identify Ty5 insertions within the rDNA. Genomic DNA was extracted from individual His⁺/5-FOA⁺ strains, each representing an independent Ty5 integration event. Insertions within the rDNA were detected by PCR with three Ty5-specific primers and four rDNA primers, the latter of which were complementary to the same DNA strand. Two of the Ty5 primers amplify an internal portion of the element; this served as a control for the PCR reaction and demonstrated that Ty5 was present in the genome. An amplification product other than the Ty5 control fragment indicated an insertion within the rDNA. Additional PCR reactions with individual rDNA primers made it possible to pinpoint the integration site. (Open boxes) 18S, 5.8S, 25S, and 5S rRNA genes. The direction of transcription is indicated. (ABF1, RAP1) Characterized binding sites for these proteins (Buchman et al. 1988; Kang et al. 1995); (ARS) an origin of DNA replication (Miller and Kowalski 1993). Oligonucleotides used in the PCR assay are labeled by their DVO designator. (B) The location of Ty5 insertions within the rDNA. (Vertical arrows) Sites of insertion; (arrows with circular tails) the three rDNA insertions identified from the 100 wild-type strains in Table 6. All others represent the 29 insertions identified in *sir4-42* (Table 6). (Arrows pointing down) Insertions in the same 5' to 3' orientation as the 35S rDNA; (arrows pointing up) insertions in the opposite orientation.

The Sir complex and Ty5 cDNA recombination

In addition to their effect on Ty5 integration specificity, loss of Sir proteins influenced Ty5 cDNA recombination. We measured Ty5 integration or recombination by the His⁺ phenotype that results when Ty5 cDNA joins a plasmid or chromosomal substrate. Deletions of each of the *SIR* genes increased the overall frequency of His⁺ cell formation, and this increase was due to an increase in cDNA recombination. For $\Delta sir1$, $\Delta sir2$, and $\Delta sir3$ strains, plasmid recombination increased from 1.7- to 2.3-fold. Loss of Sir proteins results in the expression of the silent mating loci, and we previously observed comparable increases in cDNA recombination in strains with silencer mutations that express both α - and α -mating-type information (Zou and Voytas 1997). In this study we assayed recombination in Δsir strains that expressed only α -mating-type information, and we found that most of the increase in cDNA recombination in the Δsir single mutants was due to the change in the expression of mating-

type information. Integration also decreased slightly in $\Delta sir1$, $\Delta sir2$, and $\Delta sir3$ strains. Shifts in the balance of integration and recombination have been observed for Ty5 and Ty1 as a consequence of mutations in integrase (Sharon et al. 1994; Ke and Voytas 1999). In the Δsir strains, it is possible that cDNA is produced more efficiently or that more cDNA is diverted to the recombination pathway.

A particularly pronounced effect on cDNA recombination was observed for the $\Delta sir4$ strain. It showed a >10-fold increase in the frequency of His⁺ cell formation, of which 91% of the events were due to plasmid recombination, 5.7% to chromosomal recombination, and only 3.3% were integration events. This contrasts with wild type, in which 38% of the His⁺ cells were due to plasmid recombination, 3.1% to chromosomal recombination and 58.9% to integration. The high frequency of His⁺ cell formation in $\Delta sir4$ appears to be the result of contributions made by both the expression of the mating-type loci and the loss of Sir4p. Although the mechanism by which this occurs cannot yet be ascertained, possibilities include a general increase in homologous recombination, inhibition of cDNA degradation, an increase in cDNA synthesis, or an alteration in the structure of the integration complex that makes more cDNA available for recombination. Recently, the Sir complex has been found to play a role—either directly or indirectly—in nonhomologous end joining (NHEJ) (Astrom et al. 1999; Martin et al. 1999; Mills et al. 1999). The mammalian NHEJ pathway requires Ku70 and Ku80, DNA-binding components of the DNA-dependent protein kinase, which are required to complete retroviral integration (Daniel et al. 1999). Sir4p physically interacts with Hdf1p—the yeast Ku70 homolog (Tsukamoto et al. 1998), and this may explain the decrease in integration efficiency observed in Δsir backgrounds. The precise mechanism by which Sir4p and the other Sir proteins impact Ty5 homologous cDNA recombination and integration is the subject of ongoing experimentation.

rDNA targeting in *sir4-42*

If the model for retroelement target specificity is correct, namely that integration complexes interact with chromatin, then changes in chromatin states that occur during the onset of developmental programs would be predicted to impact target specificity. The Sir complex undergoes a dramatic reorganization at the onset of senescence. Immunofluorescence studies have demon-

Table 6. Targeting efficiency of Ty5 to rDNA

Genotype	Chromosomal events characterized	Number of rDNA insertions	Percent rDNA insertions
$\Delta rad52$	100	3	3.0%
$\Delta sir2 \Delta rad52$	102	6	5.9%
$\Delta sir4 \Delta rad52$	94	6	6.4%
<i>sir4-42</i> $\Delta rad52$	113	29	26%

strated that Sir3p and Sir4p move from the telomeres to the rDNA (nucleolus) as yeast cells age (Kennedy et al. 1997). Like in old cells, Sir3p and Sir4p are constitutively located within the nucleolus in strains carrying the *sir4-42* allele.

We have shown that Ty5 target specificity is dramatically altered in *sir4-42* strains. Only 3% of Ty5 integration events occur within the rDNA in wild-type strains, and this increases to 26% in *sir4-42* strains. Not only does Ty5 follow the movement of the Sir complex, but also the pattern of integration events within the rDNA suggests how this chromatin is organized. Ty5 insertions predominantly occur at two locations within the rDNA—one near the 5S rRNA gene and the other near the junction of the 5.8S and 25S genes. These regions are roughly coincident with binding sites for three proteins or protein complexes that assemble at the subtelomeric X repeats and the *HM* silencers (Fig. 5). ORC and Abf1p have been shown to bind on either side of the 5S gene (Miller and Kowalski 1993; Kang et al. 1995), and the 25S integration hotspot is largely coincident with a known Rap1p-binding site (Buchman et al. 1988). Although we cannot exclude a role for other rDNA-associated proteins, the distribution of Ty5 insertions suggests that the same proteins responsible for the assembly of the Sir complex at the telomeres and mating silent loci may also nucleate the assembly of the Sir complex at the rDNA. Chemical cross-linking of Sir2p to the rDNA has shown previously that it is preferentially localized in the vicinity of the 5S gene, supporting a nonrandom distribution of silencing factors within the rDNA (Gotta et al. 1997). Defining chromatin at the rDNA is an active area of investigation, particularly because of its role in aging, and as described more recently, because of its role in the cell cycle (Shou et al. 1999; Straight et al. 1999). Monitoring Ty5-targeting patterns provides a new tool for dissecting rDNA chromatin dynamics.

Approximately 74% of the insertions in *sir4-42* are not associated with the rDNA. Among these, we observed a high incidence of insertions (6/15) into or near pre-existing Ty1 or Ty3 elements. In Δ *sir4* strains, 2/9 insertions were also within or near Ty1 elements. It may be that when targeting to silent chromatin is disrupted, Ty5 defaults to a Ty1-like targeting mechanism recognizing RNA Pol III transcriptional machinery. We do not find this explanation particularly compelling, however, because insertions into Ty1 and Ty3 were not recovered in Δ *sir2* or Δ *sir3* strains, both of which have targeting defects. A second possibility is that the Ty-associated insertions in *sir4-42* strains identify other sites at which silent chromatin assembles in genetically aged cells. Because of their dispersed nature, these loci are likely difficult to observe in immunofluorescence studies of Sir complex localization, and Ty5 therefore may be a more sensitive tool for monitoring chromatin changes. Aging is typically associated with a decrease in translational efficiency (Sinclair et al. 1998), and alterations in chromatin structure may be associated with genes other than the rDNA that encode components of the translational apparatus. Candidates include tRNA genes, which are

Ty1 and Ty3 integration hotspots and may explain the observed targeting pattern in *sir4-42*. Recently, it has been shown that a Ty1 LTR and tRNA gene can serve as boundary elements that delimit silent chromatin at the *HMR* locus (Donze et al. 1999). Alternatively, Ty5 may sense changes in chromatin assembled at native Ty insertions. A more exhaustive survey of insertions in *sir4-42* strains will be required to identify significant targeting trends and to further develop these alternative hypotheses. Nonetheless, the data presented here indicate that Ty5, and retrotransposons in general, may be powerful tools for dissecting the mechanisms underlying chromatin remodeling.

Materials and methods

Strains

The genotypes and sources of the yeast strains used in this study are listed in Table 7. YSZ269, which carries the *sir4-42* allele, was constructed by integrating plasmid pSZ289 at the *TRP1* locus of JRY4582 (gift of Jasper Rine, University of California, Berkeley). pSZ289 was constructed by first cloning a *SacII/ClaI* fragment of *SIR4* from pRS316-*SIR4* (gift of Jasper Rine, University of California, Berkeley) into pRS404 (Sikorski and Hieter 1989). This generated plasmid pSZ268. PCR was carried out with primers DVO408 and DVO409 to amplify the carboxyl terminus of *SIR4*. Primer DVO408 generates a stop codon at amino acid 1238, which is the mutation found in the *sir4-42* allele (Kennedy et al. 1995). The amplification product was cloned, and the entire sequence was confirmed by DNA sequencing. The mutagenized carboxyl terminus was then used to replace the wild-type fragment of pSZ268, generating pSZ286. A transcriptional terminator from *MRP2* (Peterson and Myers 1993) was inserted into the *SalI/BglIII* sites downstream of the *sir4-42* carboxyl terminus to create pSZ289. One-step gene disruption was used to make all *RAD52* deletions, with plasmids pSM20 (*TRP1* marker) or pMS21 (*LEU2* marker) (gift of L. Prakash and S. Prakash, University of Texas, Galveston). Gene disruptions were confirmed by PCR with primers DVO392 and DVO393, which are complementary to the *RAD52* gene. *HMR* deletion strains were constructed by one-step PCR gene disruption with primers DVO1066 and DVO1069. *HMR* deletions were confirmed by PCR and mating tests.

Integration and recombination assays

All yeast strains carry the plasmid pNK254, which has a Ty5 element under transcriptional control of the *GAL1-10* promoter and a *his3AI* selectable marker gene (Ke and Voytas 1997). Yeast cells were initially grown as patches on synthetic complete medium without uracil and with glucose (SC-U + glucose) at 30°C for 2 days. Patches were subsequently replica plated to complete media without uracil and with galactose (SC-U + galactose) to induce transposition and were grown at 23°C for an additional 2 days. To calculate transposition efficiency, cells were scraped from the plates and resuspended in 1 ml of sterile water. For each patch, 100 μ l of a 10^5 -fold dilution was plated onto non-selective medium (YPD) to calculate the total cell number. A total of 100 μ l of the original suspension (or a 10-fold dilution) was plated onto selective medium without histidine (SC-H) to select cells with Ty5 cDNA integration or recombination events. The frequency of His⁺ cell formation was calculated as the ratio of the number of colonies on SC-H plates to the num-

Table 7. Yeast strains and oligonucleotides used in this study

Strain	Genotype	Source
W303-1B	<i>MATα ade2-1 can 1-100 his3-11,15 leu2-3 trp1-1 ura3-1</i>	Alan Myers
AMR26	W303-1B <i>sir1::LEU2</i>	Rolf Sternglanz
YDS71	W303-1B <i>sir2::TRP1</i>	Rolf Sternglanz
YDS73	W303-1B <i>sir3::LEU2</i>	Rolf Sternglanz
YAB61	W303-1B <i>HMR-E aeb</i>	Rolf Sternglanz
JRY4582	W303-1B <i>ADE2 Δlys2 sir4::LEU2</i>	Jasper Rine
YSZ269	JRY4582 <i>TRP1 sir4-42</i>	this study
YYZ1	W303-1B <i>rad52::TRP1</i>	this study
YYZ3	YDS71 <i>rad52::LEU2</i>	this study
YYZ4	RS862 <i>rad52::LEU2</i>	this study
YYZ5	JRY4582 <i>rad52::TRP1</i>	this study
YYZ48	YSZ269 <i>rad52::TRP1</i>	this study
YYZ212	JRY4582 <i>hmr::TRP1</i>	this study
YYZ213	AMR26 <i>hmr::TRP1</i>	this study
YYZ214	YDS71 <i>hmr::LEU2</i>	this study
YYZ215	YDS73 <i>hmr::TRP1</i>	this study

Oligo	Sequence	Gene
DVO208	5'-CAGCCGGAATGCTTGGCCA-3'	<i>HIS3</i>
DVO308	5'-AGATCTCACCATTGAAAGGCTTCG-3'	<i>SNF6</i>
DVO309	5'-ATCGATGAACGGCTCATTTGGAATAA-3'	<i>SNF6</i>
DVO392	5'-ATCTCCAAGAGAGTTGCGG	<i>RAD52</i>
DVO393	5'-GCCTCAGCAGGTGCGGCC	<i>RAD52</i>
DVO495	5'-CCATAGTTTCTGTGTACAAGAGT-3'	Ty5 LTR
DVO496	5'-CTTGTCTAAAACATTACTGAAACAAT-3'	Ty5 LTR
DVO583	5'-GGACAAATAGTGATTTCAGCCGTC-3'	Ty5 <i>GAG</i>
DVO247	5'-CACGAGCTCATCTAGAGCC-3'	<i>HML-E</i>
DVO212	5'-ACCAGAGAGTGTAACAACAG-3'	<i>HMR-E</i> and <i>HML-E</i>
DVO251	5'-TGCTGAAGTACGTGGTGAC-3'	<i>HMR-I</i> and <i>HML-I</i>
DVO252	5'-TTCTCGAAGTAAGCATCAAC-3'	<i>HML-I</i>
DVO211	5'-TGGTAGAAGCAGTAGTAAC-3'	<i>HMR-E</i>
DVO253	5'-AGCCCTATTTCGCGTCGTG-3'	<i>HMR-I</i>
DVO1066	5'-CCCGACTATGCTATTTTAAATCATTGAAAACGAA TTTATTTAGATTGTACTGAGAGTGAC-3'	<i>HMR</i>
DVO1069	5'-TATCGTCATATACAAATCTAGAAATTACCAGAG CTATCCACTGTGCGGTATTTTCACACCG-3'	<i>HMR</i>
DVO1070	5'-TACGAAGATTCTCGATTCCG	<i>HMR</i>
DVO1079	5'-TTACTGTTGCGGAAAGCTGA	<i>HMR</i>
DVO553	5'-CACCCACTACACTACTCGGTCAGG-3'	rDNA
DVO554	5'-ATCTGGTTGATCCTGCCAGTAGTC-3'	rDNA
DVO555	5'-TCTGTTTGGTAGTGAGTGATACTC-3'	rDNA
DVO556	5'-AGACCCTGTTGAGCTTGACTCTAG-3'	rDNA
DVO575	5'-TTCCGCACCTTTTCTCTGTCCAC-3'	rDNA
DVO576	5'-AAGACCCGAATGGGCCCTGTATCG-3'	rDNA
DVO577	5'-AGAGGGCACAAAACACCATGTCTG-3'	rDNA
DVO578	5'-TATCCGAATGAACTGTTCTCTCG-3'	rDNA
DVO408	5'-GGACTAGTTAAGTACCAGTTTGTGAATGT-3'	<i>sir4-42</i>
DVO409	5'CCCAAGCTTACAGAACAAGCCTGATGCA-3'	<i>SIR4</i>

ber of colonies on YPD plates adjusted by the dilution factor. To identify plasmid recombinants, His⁺ colonies were streaked onto SC-H medium with 5-fluoroorotic acid (SC-H + 5-FOA). 5-FOA selects against the *URA3* gene (Boeke et al. 1987), and plasmid recombinants fail to grow on SC-H + 5-FOA medium because both the *URA3* and *HIS3* markers are carried on the same plasmid. The frequency of His⁺ cells with chromosomal Ty5 insertions was calculated as the ratio of the number of colonies on SC-H + 5-FOA to the number of colonies on YPD plates.

To distinguish between chromosomal recombination and integration events, yeast colony PCR was carried out with the

His⁺/5-FOA⁺ strains (Ausubel et al. 1987). Integration events were identified with a Ty5-specific primer (DVO496) and an *HIS3*-specific primer (DVO208). A specific 0.6-kb fragment was amplified from genomic DNA of cells with Ty5 integration events; no such band was amplified from DNA of His⁺/5-FOA⁺ cells that had undergone gene conversion of *his3-11,15*. Another pair of primers (DVO308 and DVO309), that amplify a 0.9-kb fragment of the *SNF6* gene served as a PCR control. Amplifications were performed for 30 cycles by the following program: 94°C for 1 min, 48°C for 1 min, and 72°C for 2 min, with a final extension step of 72°C for 10 min. Percent integration was calculated by dividing the number of the samples with both

the 0.6-kb Ty5 fragment and the 0.9-kb *SNF6* control fragment by the total samples with the 0.9-kb *SNF6* fragment. The overall transposition efficiency was calculated as the frequency of His⁺ cells with Ty5 on the chromosome times the percent integration.

Mapping chromosomal Ty5 integration events

To obtain strains with independent Ty5 integration events, His⁺ wild-type or Δsir strains were generated by the above transposition protocol. Chromosomal integration events were identified by the PCR method described above and confirmed by Southern blot analysis (Ausubel et al. 1987). Filters were hybridized with radiolabeled Ty5 integrase, LTR and *HIS3* probes. His⁺/5-FOA⁺ strains carrying one integrase band, two LTR bands, and two *HIS3* bands were considered integration events. His⁺/5-FOA⁺ strains with no integrase band and no LTR bands and only one *HIS3* band were considered to have arisen by gene conversion of the endogenous *his3-11,15* allele by the *HIS3* marker on the Ty5 cDNA. Sequences flanking the Ty5 integration sites were amplified as described previously by inverse PCR (Zou et al. 1996a), sequenced, and mapped onto the complete *S. cerevisiae* genome sequence (Cherry et al. 1997).

A PCR Assay to monitor Ty5 integration near the HM loci

Pools of genomic DNA were prepared from 10 His⁺/5-FOA⁺ strains representing 10 independent chromosomal events (Ausubel et al. 1987). The majority of His⁺/5-FOA⁺ strains were assumed to have only one Ty5 element as supported by the previous characterization of >70 His⁺/5-FOA⁺ strains (Zou et al. 1996a,b; Zou and Voytas 1997). Integration events near the HM loci were identified and confirmed by PCR screening with four groups of four primers. Each group included two Ty5 LTR-specific primers (DVO495 and DVO496) and two primers that amplified a ~2-kb window flanking one of the silencers (*HML-E*, DVO247 and DVO212; *HML-I*, DVO251 and DVO252; *HMR-E*, DVO211 and DVO212, or *HMR-I*, DVO251, and DVO253). Because DNA from most of the strains in each reaction did not have a Ty5 element near the tested silencer, the presence of the ~2-kb band served as a control for the PCR reaction. Amplifications were performed with 50 ng of the pooled genomic DNA for 30 cycles by the following program: 94°C for 1 min, 48°C for 1 min, and 72°C for 3 min, with a final extension step at 72°C for 10 min.

A PCR assay to monitor Ty5 integration within the rDNA

Genomic DNA was prepared from individual His⁺/5-FOA⁺ strains, each representing independent chromosomal integration events. Insertions within the rDNA were identified and confirmed by PCR screening. Each reaction included three Ty5-specific primers (DVO495, DVO496, and DVO583) and four primers complementary to the same rDNA strand (Group 1: DVO553, DVO554, DVO555, DVO556; Group 2: DVO575, DVO576, DVO577, DVO578). The entire 9.1-kb rDNA repeat was covered by the eight rDNA primers so that any Ty5 insertion within the rDNA could be identified by two rounds of PCR. The amplification of a 1.6-kb Ty5 fragment by DVO583 and DVO495 served as a control for the PCR reactions and indicated the presence of Ty5 in the genome. Once an insertion was identified, additional rounds of PCR were conducted with individual Ty5 and rDNA primers to confirm its presence and to map it within the rDNA. Amplifications were performed with 50 ng of DNA for 30 cycles by the following program: 94°C for 1 min,

60°C for 1 min, and 72°C for 2 min, with a final extension step at 72°C for 10 min.

Acknowledgments

We thank R. Sternglanz, J. Rine, and A. Myers for providing strains used in this study. This work was funded by American Cancer Society Grant VM145 to D.F.V. This is Journal Paper No. J-18446 of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA, Project No. 3383, and was supported by Hatch Act and State of Iowa Funds.

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Yunxia Zhu, Sige Zou, David A. Wright, et al.

Genes Dev. 1999, **13**:

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