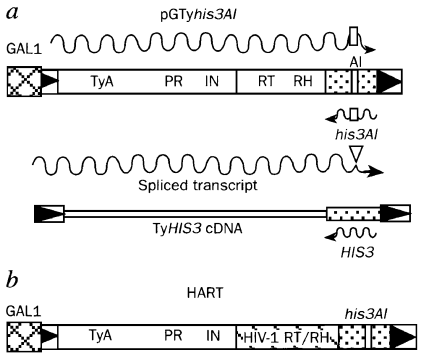
HIV reverse transcription in yeast

SIR — The study of human immunodeficiency virus (HIV) reverse transcriptase/ribonuclease H (RT/RH) and other medically important reverse transcriptases would be greatly enhanced by simple, safe and inexpensive *in vivo* assays. We combined the reverse transcription indicator gene *his3AI* with hybrid Ty/HIV elements to demonstrate that HIV-1 RT/RH can substitute for the RT/RH of the Ty*1* retrotransposon in *Saccharomyces cerevisiae*.

S. cerevisiae strains harbour retrotransposons (Ty elements) which replicate like retroviruses[1]. Ty encodes an RT/RH necessary for replicative transposition and an integrase required for insertion at novel sites. The plasmid pGTy*his3AI* (*a* in the figure) was developed for analysis of retrotransposition[2]. Splicing and reverse transcription of the galactose-inducible Ty*his3AI* messenger RNA yields Ty complementary DNA carrying a functional *HIS3* gene that can be integrated into the genome. Ty cDNA can also undergo homologous recombination with endogenous Ty elements. Both transposition and cDNA recombination result in His$^+$ cells.



The *his3AI* indicator gene and hybrid Ty*1*/HIV-1 RT/RH elements. *a*, pGTy*his3AI*, the inducible galactose-promoter-driven Ty*1* element marked with *his3AI* (ref. 2), serves as an indicator of passage through an RNA intermediate and reverse transcription. Ty RNA (wavy line), carrying an antisense copy of *his3AI* containing a spliceable artificial intron (AI; open rectangle) interrupting *his3*, is first spliced and then reverse-transcribed, generating Ty*HIS3* cDNA. Integration or homologous recombination[7] of this cDNA results in histidine prototrophy. *b*, The RT/RH domain of HIV-1 was used to replace Ty*1* RT/RH, resulting in HART elements.

Hybrid Ty/HIV-1 elements (HARTs; *b* in the figure) contain HIV-1 RT/RH adjacent to the Ty protease cleavage site separating Ty*1* integrase and RT/RH, completely replacing Ty RT/RH. Yeast expressing these elements produce HIV-1 RT/RH as a protein of relative molecular mass 66,000, suggesting that processing is efficient (D.V. N. and S. Moore, unpublished results). The *in vivo* activity of the HARTs is readily detected in strains defective in endogenous Ty expression (*spt3*; ref. 3) by the production of His$^+$ cells (see table). Frameshift mutation of the HIV-1 open reading frame, or point mutations that disrupt either the polymerase or RNase H activities of HIV-1 RT/RH (ref. 4), decrease the production of His$^+$ cells by 100–500-fold. Residual activity in the RT/RH mutants probably reflects low-level expression of endogenous Ty elements and can be further reduced by performing the assay at 30 °C, when Ty transposition, but not the activity of the Ty/HIV-1 hybrids, is inhibited[5].

Ty transposition occurs by integrase activity, is independent of homologous recombination and, indeed, occurs in cells (*rad52*) incapable of homologous recombination[6,7]. In contrast, the HART elements are dependent on *RAD52* for production of His$^+$ cells (see table), suggesting that the cDNA produced by HIV-1 RT/RH is not a substrate for Ty integrase. It is, in fact, unlikely that HIV-1 RT/RH recognizes the Ty signals (polypurine tract and transfer RNA primer binding site) necessary to generate a complete element with correct ends for transpositional integration. We propose that HART cDNAs recombine into the genome by homology with endogenous Ty elements, as has been demonstrated for integrase-defective Ty elements and Ty elements that cannot produce correctly processed ends[7].

HIV-1 RT/RH HYBRID ELEMENTS ARE ACTIVE IN YEAST

		Frequency of His$^+$		
		20°C		30°C
	HIV RT/RH	*spt3 RAD52*	*spt3 rad52*	*spt3 RAD52*
Ty	–	2×10^{-2}	7×10^{-2}	
HART 1	Wild type	6×10^{-3}	7×10^{-5}	
HART 1-m	Frameshift	1×10^{-5}	8×10^{-6}	
HART 21	Wild type	1.8×10^{-3}		2.7×10^{-3}
HART 22	pol$^-$	6.7×10^{-6}		$<3.0\times10^{-7}$
HART 23	rh$^-$	1.6×10^{-5}		$<1.5\times10^{-7}$

HIV-1 RT/RH-mediated reverse transcription was monitored by selection of histidine prototrophy following the induction of HART expression as described[2]. The frequency of HART-mediated histidine prototrophy were determined in *RAD52* (DG1251: MATα *ura3-167 trp1-GB spt3-101 his3Δ200*) and *rad52* (DG1286: MATα *ura3-167 trp1-GB spt3-101 his3Δ200 rad52-GB*) strains at 20 °C and 30 °C.

Although an *Escherichia coli*-based *in vivo* assay for the DNA-dependent DNA polymerase activity of HIV-1 RT/RH has been described[8], our HARTs provide the first efficient *in vivo* assays for RNA-dependent polymerase and RNase H activities of HIV-1 RT/RH outside the intact virus. Screening for drugs that inhibit the RT/RH of HIV-1 or other retroviruses with a simple genetic assay in yeast is therefore possible. This assay may also be useful in characterizing drug-resistant variants of HIV-1 RT/RH.

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Why are toads right-handed?

SIR — We were pleased to see the report by Bisazza *et al.*[1] indicating that toads are preferentially right-handed. Much of Bisazza *et al.*'s data came from tests where toads preferentially used their right hand to wipe away material stuck to their head and face. We offer here a simple adaptive explanation for why these animals preferentially used that hand for this grooming activity.

Anurans have strong emetic reflexes[2]. Toxic material in the stomach provokes vomiting, and when anurans vomit, they not only regurgitate stomach contents but also the stomach itself[3]! Because the anuran stomach is asymmetric — with a shorter mesentery on the right than the left side — the stomach is tethered to that side. Consequently, the prolapsed stomach always hangs out the right side of the mouth[3].

We have watched and videotaped emesis in anurans from many different families and genera[2], including *Bufo*. In a stereotypic fashion, frogs and toads wipe away remaining vomitus from the surface of the prolapsed stomach before re-swallowing it[3]. Any anuran that does

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