Inhibition of a Yeast LTR Retrotransposon by Human APOBEC3 Cytidine Deaminases

James A. Dutko, Alexandra Schäfer, Alison E. Kenny, Bryan R. Cullen, and M. Joan Curcio

Supplemental Experimental Procedures

Plasmid Construction

Mammalian expression plasmids encoding HA-tagged forms of β-ARR-2, hA1, hA3C, hA3G, hA3F, and mA3 have been previously described [S1, S2]. The M1, M2, and M1M2 mutants of hA3G were obtained by PCR-mediated mutagenesis. Yeast expression plasmids encoding HA-tagged APOBEC3 proteins were constructed by excision of each cDNA as a HindIII/XhoI fragment and insertion into pYES2 (Invitrogen). Plasmids encoding Ty1, MS2 coat protein, or HIV-1 Gag linked to a carboxy-terminal GST moiety were generated with the pK vector [S3].

Transposition Assay

Three independent transformants of plasmid pYES2 or pYES2 containing an APOBEC3 insert in strain JC3212 (*MAT*a, *ura3Δ0, his3Δ1, leu2Δ0, met15Δ0, Ty1his3AI[Δ1]-3114*), JC297 (*MAT*α, *ura3-167*, *his3Δ200*, *trp1::hisG*, Ty1*his3AI*-270), or JC560 (*MAT*α, *ura3-167*, *his3Δ200*, *trp1::hisG*, Ty2*his3AI*-1) were grown in SC-Ura 2% glucose broth at 30°C. Cells were diluted 1:100 in SC-Ura 2% galactose broth and grown for 48 hr at 20°C. An equal volume of 2×YPD broth was added and cultures were grown for 24 hr at 20°C. Aliquots of each culture were plated on SC-Ura 2% glucose agar and SC-Ura-His 2% glucose agar and incubated at 30°C. The frequency of Ty1*his3AI* transposition is the number of His^+ Ura^+ colonies divided by the total number of Ura^+ colonies plated.

VLP Purification

Strain JC3212 harboring pYES2 or pYES2-APOBEC3G-HA or an *spt3* strain harboring pYES2-APOBEC3G-HA was grown in 500 ml SC-Ura 2% galactose broth at 20°C to an OD_{600} of 0.8, and the lysate was fractionated on a 20%/30%/45%/70% sucrose step gradient as previously described [S4]. The 45% fraction at the 75% interface was pelleted and resuspended in 700 ul buffer A (10 mM HEPES-KOH [pH 7.8], 15 mM KCl, 5 mM EDTA) plus 10% sucrose and loaded onto a 11.5 ml 20%–60% continuous sucrose gradient. Gradients were centrifuged for 3 hr at 25,000 rpm in a Beckman SW41 rotor. Fractions of 0.5 ml were collected across the gradient. Proteins in the first and then every third fraction were analyzed by Western blotting with rabbit anti-TyA1 antiserum [S4] or mouse monoclonal anti-HA antibodies (Santa Cruz).

GST Pulldown

293T cells were cotransfected with 1.5 μg of phA3G-HA or pβ-ARR-2-HA DNA together with 1.5 μg of pK/HIVGAG-GST, pK/TY1GAG-GST, pK/MS2-GST, or pK/GST DNA. Plasmid pDM128/4XMS2 DNA (1 μg) was cotransfected with the pK/MS2-GST DNA. Forty-eight hours after cotransfection, the cells were lysed in 250 μl of lysis buffer (50 mM TRIS-HCL [pH 7.4], 150 mM NaCl, 5mM EDTA, 5% glycerol, 1% Triton X-100). A 200 μl aliquot of the clarified lysate was incubated with glutathione-Sepharose beads for 1 hr at 4°C. In some experiments, the lysate was incubated with RNase A prior to addition of the beads. After incubation, the beads were washed three times with lysis buffer, and the bound proteins analyzed by Western blotting with mouse monoclonal antibodies specific for the HA epitope (Covance). Aliquots of input lysate were also subjected to Western blot analysis with anti-HA antibodies or with mouse monoclonal anti-GST antibodies (Santa Cruz).

Integration Assay

Strain JC3212 harboring plasmid pYES2 or derivatives was grown at 20°C for 2 days in SC-Ura 2% galactose broth. The *tec1*Δ strain was grown at 20°C for 2 days in SC 2% galactose broth. Isolation of genomic DNA, PCR-reaction conditions and the TYBOUT-2 and SUF16-2 primers were as described [S5]. PCR reactions containing 300 ng of template DNA were incubated at an annealing temperature of 65°C for five cycles followed by a variable number of cycles at an annealing temperature of 60°C. Control reactions containing one of the two primers were incubated for a total of 35 cycles. PCR products were visualized on agarose gels stained with ethidium bromide.

Sequencing of Ty1*HIS3* cDNA

Genomic DNA was prepared from cultures seeded with single-colony-purified His^+ strains. The DNA was subject to PCR amplification with primers HIS3HOPS (5′-CGCAGTTGTCGAACTTGGTTTGCA AAG-3′) and TYBRT-S (5′-AACTTAGCGCTCCAGGTCAAC-3′). PCR products were obtained from His^+ strains harboring Ty1*HIS3* and Ty1*his3AI*, but not from His^- control strains harboring only Ty1*his3AI*. Approximately 700 nucleotides of Ty1 *pol* were sequenced from PCR products with primer HIS3END-S (5′-ATGTTCCCTCCAC CAAAGGTG-3′). Ty1 sequence was compared to the sequence of Ty1-H3 (M18706). Two of 19 Ty1*HIS3* PCR products derived from strains that expressed hA3G had a large number (39 and 11) of clustered nucleotide substitutions. The sequences of these two Ty1 cDNAs exactly matched the sequences of genomic Ty1 elements in the *Saccharomyces* Genome Database (www.yeastgenome.org), indicating that the nucleotide changes arose by template switching during reverse transcription or by repair after integration, with genomic copies of Ty1. Because we could not determine if these two Ty1*HIS3* cDNAs had been subject to editing, they were eliminated from our analysis.

Supplemental References

S1. Oakley, R.H., Laporte, S.A., Holt, J.A., Caron, M.G., and Barak, L.S. (2000). Differential affinities of visual arrestin, beta arrestin1, and beta arrestin2 for G protein-coupled receptors delineate two major classes of receptors. J. Biol. Chem. 275, 17201–17210.

S2. Wiegand, H.L., Doehle, B.P., Bogerd, H.P., and Cullen, B.R. (2004). A second human antiretroviral factor, APOBEC3F, is suppressed by the HIV-1 and HIV-2 Vif proteins. EMBO J. 23, 2451–2458.

S3. Brownawell, A.M., and Macara, I.G. (2002). Exportin-5, a novel karyopherin, mediates nuclear export of double-stranded RNA binding proteins. J. Cell Biol. 156, 53–64 Published online January 3, 2002. 10.1083/jcb.200110082

S4. Conte, D., Barber, E., Banerjee, M., Garfinkel, D.J., and Curcio, M.J. (1998). Posttranslational regulation of Ty1 retrotransposition by mitogen-activated protein kinase Fus3. Mol. Cell. Biol. 18, 2502–2513.

S5. Scholes, D.T., Banerjee, M., Bowen, B., and Curcio, M.J. (2001). Multiple regulators of Ty1 transposition in *Saccharomyces cerevisiae* have conserved roles in genome maintenance. Genetics 159, 1449–1465.