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Transposition of a Ty3 *GAG3-POL3* Fusion Mutant Is Limited by Availability of Capsid Protein

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Ty3 encodes structural proteins in its upstream open reading frame (*GAG3*) and catalytic proteins in an overlapping open reading frame (*POL3*). As is the case for retroviruses, high levels of structural protein versus catalytic proteins are synthesized and we show here that catalytic proteins are derived from a *GAG3-POL3* fusion polyprotein. To evaluate the relative contributions of structural and catalytic components of the Ty3 particle, we perturbed the balance of these proteins by fusing the *GAG3* and *POL3* frames. This fusion Ty3 was capable of complementing low levels of transposition of a donor Ty3 which contained only *cis*-acting sequences required for transposition. Examination of extracts of cells expressing the *GAG3-POL3* fusion mutant showed that particle formation differed qualitatively and quantitatively from viruslike particle formation by wild-type Ty3. Surprisingly, expression of 238 codons of *GAG3*, encoding only capsid protein, complemented transposition and particle formation defects of the fusion mutant, showing that the limiting deficiency was in capsid, and not in nucleocapsid, function. In addition, protein containing the capsid domain expressed alone accumulated in the same particulate fraction as viruslike particles, showing that it was sufficient for particle formation. The activity of the Ty3 fusion mutant contrasts with the inviability of mutant retroviruses in which *gag* and *pol* frames were fused and argues that retrotransposons tolerate considerable variation in the nucleoprotein complexes that permit replication and integration.

Formation of particles within which replication occurs appears to be a universal property of retroviruses and related retrotransposons (19). Two classes of retrotransposons, copia-like and gypsy-like elements, are represented in *Saccharomyces cerevisiae* by Ty1, Ty2, and Ty4 and by Ty3, respectively (4). These classes of elements differ in transcriptional regulation and in the *cis*-acting sequences required for transposition. The catalytic proteins required for transposition are very distantly related, and the order in which they are encoded differs between the classes. The structural proteins encoded by the two classes appear to be unrelated. High-level expression of each class has been shown to result in formation of intracellular viruslike particles (VLPs). This investigation was undertaken to explore the mechanism which controls the differential expression of structural and catalytic components of the Ty3 VLP and to determine how sensitive particle formation and transposition are to perturbation of this ratio. Ty3 is 5.4 kbp long and is composed of 340-bp long terminal repeats (LTRs), termed sigma elements, flanking an internal domain. Ty3 transcription begins in the upstream LTR and terminates in the downstream LTR. The transcript is terminally redundant and polyadenylated and contains regions analogous to the retrovirus minus- and plus-strand priming sites (10). Ty3 proteins are encoded in two open reading frames (ORFs), *GAG3* of 307 codons, predicted to encode a protein of 290 amino acids, and *POL3*, which contains 1,270 codons (21, 22). *POL3* overlaps *GAG3* by 38 nucleotides (nt) in the plus-one frame. Haploid cells which are not induced with pheromones produce little or no Ty3 RNA (10). However, if Ty3 is cloned under control of a heterologous upstream activating sequence (UAS), high levels of Ty3 transcription can be induced over prolonged periods (21). Cells in which Ty3 has been induced in this manner contain VLPs of approximately 156S (20). The VLP fraction contains the 5.2-kb Ty3 transcript, Ty3 DNA, and Ty3-encoded proteins. The *GAG3* proteins of the nucleoprotein complex have apparent masses of 39, 38, 31, 26, and 9 kDa. The 26-kDa capsid protein (CA) contains a portion of the major homology region identified in retroviral CA proteins (49) and is encoded at the upstream end of *GAG3*. The 9-kDa nucleocapsid protein (NC) contains one copy of the $CX_2CX_4HX_4C$ motif found in all retroviral NC proteins and is encoded in the downstream portion of *GAG3*. The 39- and 38-kDa species react with antibodies raised against peptides from the amino- and carboxyl-terminal regions. These properties and sizes would be consistent with translation products encoded by the complete *GAG3* ORF. The 31-kDa protein reacts with the antibody raised against the amino-terminal peptide but not with that raised against the carboxyl-terminal peptide. The *POL3* gene encodes an aspartyl protease (PR) with an apparent mass of 16 kDa (28), a reverse transcriptase (RT) of 55 kDa, and integrase (IN) proteins of 61 and 58 kDa. VLPs accumulate and transposition occurs when transcription of the Ty3 RNA is induced (20, 21).

Studies of expression of retroviral proteins have shown that the correct ratio of *gag* to *pol* proteins is important for infectious particle formation (11, 17, 35, 37, 41, 47). Expression of synthetic *gag-pol* fusions results in accumulation of protein but no infectious virions. On the other hand, expression of *gag* sequences alone or in the presence of wild-type (WT) viral proteins can result in particle formation (12, 27, 46, 50). Expression of the Ty1 structural protein in the absence of catalytic proteins has also been shown to result in formation of intracellular particles (7). In the case of the *Drosophila* retrotransposon copia, structural proteins and protease are encoded by a spliced transcript formed from the

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same ORF that encodes polymerase and IN. Mutations which eliminate splicing eliminate detectable particle formation, suggesting that a high ratio of structural protein and protease to other proteins is essential for stability (54). However, because the retrotransposon life cycle is intracellular, the requirement for an excess of free structural protein is potentially less stringent for retrotransposons than for retroviruses. The current study was undertaken to address the following questions. (i) are Ty3 *POL3* proteins synthesized as part of a Gag3-Pol3 precursor? (ii) If so, is high-level production of the fusion polypeptide alone accompanied by processing, particle formation, or transposition? (iii) If the polyprotein precursor is not sufficient for these activities, what is the basis of the defect?

MATERIALS AND METHODS

Yeast and bacterial strains and culture conditions. Culturing and transformation of *Escherichia coli* and *S. cerevisiae* strains were done by standard methods (1). *S. cerevisiae* yVB110 (*MAT***a** *trp1-Δ901 gal3 ura3-52 his3-Δ200 ade2-101 lys2-1 leu1-12 can1-100* Δ Ty3), which contains no endogenous Ty3 elements (21), was used for experiments in which Ty3 transposition was monitored. Immunoblot analysis was performed on strain AGY-9 (*MAT***a** *ura3-52 his4-539 lys2-801 trp1-Δ63 leu2-Δ1 spt3*) (a gift of A. Gabriel and J. Boeke, The Johns Hopkins University, Baltimore, Md.). AGY-9 showed no expression of Ty3 proteins in this immunoblot analysis in the absence of Ty3-bearing plasmids and Ty3 induction. AGY-9 transformed with plasmids expressing Ty3 was used for VLP protein analysis because the mutation in *SPT3* reduces Ty1 expression (51). AGY-9 also produces higher levels of Ty3 proteins and presents a simpler protein pattern in particulate fractions than Ty3 null strain yVB110. Processing of Ty3 proteins is indistinguishable in the two strains (20).

Single-stranded DNA used for site-directed oligonucleotide mutagenesis was generated in *E. coli* RZ1032 [*lysA*(*61-62*) *thi-1 relA1 spoT1 dut-1 ung-1* (Tet^r) *supE44*] as described by International Biotechnologies, Inc. Other plasmid manipulations were performed by using *E. coli* HB101 [F$^-$ *hsdS20* (r_B^- m_B^-) *recA13 leuB6 ara-14 proA2 lacY1 galK2 rpsL20* (Sm^r) *xyl-5 mtl-1 supE44* λ^-].

Recombinant DNA procedures. All recombinant DNA techniques were performed essentially as described in *Current Protocols in Molecular Biology* (1). The wild-type Ty3-*lacZ* fusions were made by subcloning *Eco*RI-*Xmn*I or *Eco*RI-*Sca*I fragments of Ty3 into the *Eco*RI-*Sma*I site of *lacZ* fusion vector pWF-B7. Plasmid pWF-B7 is identical to pWF-B9 (18), except that the orientation of the *HIS3* gene is reversed. It contains a polylinker upstream of *lacZ* and bacterial ori and Amp^r sequences. It is maintained in high copy number in *S. cerevisiae* by virtue of a sequence from the 2μm episome and is selectable by *HIS3* function. The *GAG3-lacZ* fusion in plasmid pDF445 contains *GAG3* through the *Xmn*I site (59 bp after the first base of the initiator Met) fused to codon 9 of *lacZ*. The *GAG3-POL3-lacZ* fusion in plasmid pDF446 contains the Ty3 sequence through the *Sca*I site, located at position 1678 in *POL3*, fused to codon 9 of *lacZ*. For diagrams of these constructs, see Fig. 1B. Plasmid pDF447 was made from pDF446 by filling in the ends of *Bgl*II-linearized Ty3 DNA with the DNA polymerase I large fragment, followed by ligation; this insertion of 4 bp created a stop codon beginning at nt 1141 in *GAG3*. pDF448, in which the *GAG3* and *POL3* frames were fused, was made by oligonucleotide site-directed mutagenesis of a clone containing the Ty3-1 fragment extending from the *Xho*I site in the upstream LTR to the *Sal*I site in the middle of the element (see Fig. 1A). The mutagenic oligonucleotide 5′CGAGTTCAAGATCGGTTAGACTCGCCTTAC GTGCTCTAC3′ was annealed to the single-stranded DNA form of this phagmid prepared in RZ1032 from Ty3 nt positions 1254 to 1293 on the plus strand and was extended to make double-stranded DNA. This was transformed into HB101. The resulting plasmid contained a deletion of 1 nt of the Ty3 sequence in the overlap region. For its position, see Fig. 1A. The cloned, mutated *Hin*dIII-*Sca*I fragment of Ty3 was transferred into the *Hin*dIII-*Sma*I sites of a vector polylinker. The *Eco*RI fragment containing this Ty3 sequence was recloned into pWF-B7 at the *Eco*RI site with *GAG3-POL3* in frame with *lacZ* to create pDF448 (see Fig. 1B and C). The Ty3-*lacZ* fusions were all expressed under the natural Ty3 promoter.

Protein expression studies and transposition studies used galactose-inducible Ty3 elements. Fusion of the *GAL1-10* UAS upstream of the putative Ty3 TATA element was previously described for the construction of pEGTy3-1 (21). Protein expression was examined by analysis of cells expressing the low-copy-number helper and high-copy-number donor Ty3 elements described below or by analysis of cells expressing high-copy-number plasmid pEGTy3-1 or pEGTy3-1-OL (pDF433). Plasmid pEGTy3-1 contains bacterial ori and Amp^r sequences and the yeast *URA3* gene and 2μm sequences to confer a high copy number. Plasmid pEGTy3-1-OL was constructed from pEGTy3-1 by substituting the *Xho*I-*Sal*I fragment, which contained the OL single-nucleotide deletion, for the analogous fragment in pEGTy3-1. Thus, pEGTy3-1 and pEGTy3-1-OL differ only by this mutation.

Two helper plasmids, pJK311AC and pJK311AC-OL (pWT and pOL, respectively), were constructed. The Ty3 element in pEGTy3-1 was fused at position 123 in the LTR to the *GAL1-10* UAS and cloned into a pIBI20-based vector (International Biotechnologies, Inc.) carrying the yeast *TRP1* gene and yeast autonomously replicating (ARS) and centromere (CEN) sequences. To construct the *GAG3-POL3* fusion plasmid (pOL), the *Xho*I restriction fragment containing the overlap (OL) mutation was removed from pEGTy3-1-OL and substituted for the analogous fragment in pJK311AC. The two low-copy-number plasmids carrying the Ty3 helper elements differed only by this single-base-pair deletion.

Plasmids carrying galactose-inducible donor Ty3 elements were constructed from pEGTy3-1 or its derivatives. Deletions of sequences in the internal domain of Ty3 were made by removing the segment of DNA between two *Bam*HI sites engineered by oligonucleotide site-directed mutagenesis. These plasmids and internal deletions were as follows: pKO253, nt 427 to 4979; pKO254, nt 592 to 4979; pJK314, nt 1130 to 4874; pJK421, nt 3687 to 4874. A *Bam*HI fragment containing the yeast *HIS3* gene, including its promoter, was ligated into the reconstructed *Bam*HI site.

β-gal assays and determination of *lacZ* expression. Ty3 transcription was induced in cells at an optical density at 600 nm of 0.4 by treatment with α-factor (0.35 μM) for 1 h (45), and β-galactosidase (β-gal) activity was assayed on permeabilized transformants as previously described (38).

For Northern (RNA) blot analysis, total RNA was extracted by the procedure of Elder et al. (15). RNA was precipitated with ethanol, redissolved in water, denatured by reaction with glyoxal (33), fractionated by electrophoresis on a 1.1% agarose gel in 10 mM sodium phosphate buffer (pH

7.0), and blotted to nitrocellulose as described by Thomas (43). For each transformant, 20 μg of RNA was analyzed. Transcripts were probed with a fragment containing nt 1 to 6200 of the *lacZ* gene isolated from plasmid pFR109 (40) and labeled by the random primer method. The *URA3* transcripts were detected with a 1.1-kbp *Hin*dIII fragment from YIP5 (42) containing the *URA3* gene and labeled by the random primer method. The sizes of RNAs were extrapolated from a series of RNA size markers (BRL) end labeled with cytidine-3, 5′-[α-^{32}P]bisphosphate. Band intensity was quantitated by scanning with a laser densitometer.

For immunoblot analysis of Ty3-*lacZ* fusion proteins, whole-cell extracts were prepared from 30 ml of cells as previously described (38) and the proteins were separated on a Laemmli sodium dodecyl sulfate (SDS)–10% polyacrylamide gel (30) and transferred to a NitroScreen West membrane (DuPont, NEN Research Products) by the wet electrophoretic transfer method (44). The sizes of proteins were extrapolated from the protein size markers (prestained, low molecular weight; Bio-Rad). The membrane was incubated with blocking buffer (phosphate-buffered saline [137 mM NaCl, 2.7 mM KCl, 4 mM Na_2HPO_4, 1.4 mM KH_2PO_4, pH 7.2] containing 5% nonfat dry milk, 0.02% sodium azide, and 0.2% Tween 20) (23) for 1 h at room temperature, incubated with affinity-purified rabbit anti-β-gal antibodies (5 Prime-3 Prime; diluted 1:1,000) for 1 h at room temperature, agitated in wash buffer (1% Nonidet P-40, 0.5% deoxycholic acid, 0.1% SDS, 150 mM NaCl, 50 mM Tris, pH 7.5), and incubated for 1 h at room temperature with ^{125}I-labeled protein A (ICN Pharmaceuticals Inc.) in blocking buffer containing 0.1 mCi of ^{125}I-labeled protein A per ml. The membrane was then agitated in wash buffer and exposed to Kodak XAR-5 film to demonstrate the presence of antibody-protein complexes.

Ty3 transposition analysis. Transposition of *HIS3*-marked donor Ty3 elements complemented by helper Ty3 elements was monitored genetically and confirmed by Southern (DNA) blot analysis. Yeast strain yVB110 was transformed separately with each of the four donor plasmids pKO253, pKO254, pJK314, and pJK421. The transformants were then used as controls or transformed with either helper plasmid pJK311AC or pJK311AC-OL (pWT and pOL, respectively). Transformations were confirmed by analysis of plasmids rescued in *E. coli*. Two independently transformed isolates for each donor or donor-helper combination were grown for 5 days on synthetic medium lacking uracil or lacking uracil and tryptophan, respectively, to select for cells containing plasmids. Each strain was grown on galactose as a carbon source to induce, or on glucose to repress, Ty3 expression. Five independent colonies representing each original transformant were then patched onto YPD (1% yeast extract, 2% peptone, 2% glucose) and grown for 1 day to allow loss of marked donor plasmids. Patches were then replica plated onto synthetic medium lacking histidine and containing 5-fluoro-orotic acid (5FOA) to select for cells which had undergone transposition. 5FOA is toxic to cells containing orotidine 5′ monophosphate decarboxylase, the product of the *URA3* gene. Cells which survived the selection on medium containing 5FOA and lacking histidine were further examined by Southern blot analysis to determine whether there was evidence for recombination-mediated rather than transposition-mediated acquisition of Ty3 elements. Two independent His$^+$, 5FOA-resistant isolates were examined for each of two independent double transformants representing each donor-helper combination tested. DNA was extracted from each of the four isolates, digested with *Eco*RI, fractionated on a 0.8% agarose gel by electrophoresis, transferred to nitrocellulose, and probed with *HIS3*-specific, sigma-specific, and pIBI20-specific probes. After washing, each hybridized nitrocellulose filter was exposed to XAR-5 film for equal amounts of time. Data are shown for two of each of the four isolates analyzed.

VLP analysis. AGY-9 transformed with each of the indicated plasmids was grown in synthetic medium containing galactose as a carbon source to induce expression of the Ty3 element and lacking uracil or uracil and tryptophan to select for donor or donor-and-helper plasmids, respectively. Cultures were grown to an optical density at 600 nm of 0.9 to 1.1. Preparation of cell extracts and sucrose step gradient fractionation of induced and control cells were done by the method of Eichinger and Boeke (14) modified as previously described (20). Extracts representing 1 liter of the induced culture were fractionated through 26-ml sucrose step gradients (70-30-20% gradients of 5, 5, and 16 ml, respectively) or through linear 10 to 50% sucrose gradients. Four milliliters was reserved from the 70-30% interface per step gradient. These fractions were concentrated by centrifugation in a Ti 50 rotor (Beckman Instruments, Inc.) at 100,000 × *g* for 1 h at 4°C and suspended in 100 μl of 10% glycerol. Extracts from cells expressing the high-copy-number, galactose-induced Ty3 elements on pEGTy3-1 and pEGTy3-1-OL were analyzed on linear 10 to 50% sucrose gradients. Proteins present in six sets of five consecutive linear gradient fractions were pooled and processed in the manner described for the step gradient, except that pellets were suspended in 50 μl of buffer B (Mg^{2+} plus 10% glycerol). Protein concentrations were determined by the Bradford assay (5), and samples were mixed with one-third of a volume of 4× Laemmli sample buffer, boiled for 2 min, and fractionated by electrophoresis on an SDS-polyacrylamide gel. Immunoblot analysis was the same for these samples as that described above for *lacZ* samples. The anti-CA and anti-IN antibodies were raised against peptides represented within the 26-kDa CA and 61- and 58-kDa IN proteins, respectively, and affinity purified. Their use has been described previously (20, 22). The anti-CA antibody was previously designated anti-GAG1.

RESULTS

***POL3* proteins are synthesized as part of a *GAG3-POL3* fusion polyprotein, and the level of this protein is low compared with the level of *GAG3* protein synthesized.** Retroviruses synthesize catalytic proteins as fusions with structural proteins (24, 25). The copia (54) and Ty1 (2, 9, 34) retrotransposons have been shown to express catalytic proteins as fusion proteins, as has yeast double-stranded killer RNA (13). Expression of free and fused structural proteins provides a mechanism for ensuring that higher levels of structural proteins than catalytic proteins are synthesized from a single RNA genome and for localization of catalytic proteins to the capsid structure. To determine the relative levels of Ty3 *POL3* and *GAG3* proteins synthesized and to determine whether *POL3* proteins are synthesized as fusion polyproteins with *GAG3* proteins, a *lacZ* fusion strategy was employed. *GAG3* and *GAG3-POL3* were fused in frame to codon 9 of *lacZ* on high-copy-number plasmids (Fig. 1). Plasmids containing Ty3-*lacZ* fusions were transformed into Ty3 null strain yVB110, and Ty3 expression was induced by treatment with α-factor. The level of β-gal activity produced from the *GAG3-POL3* fusion was about 4.3% of that of the *GAG3* fusion (Table 1). This level of *POL3* expression

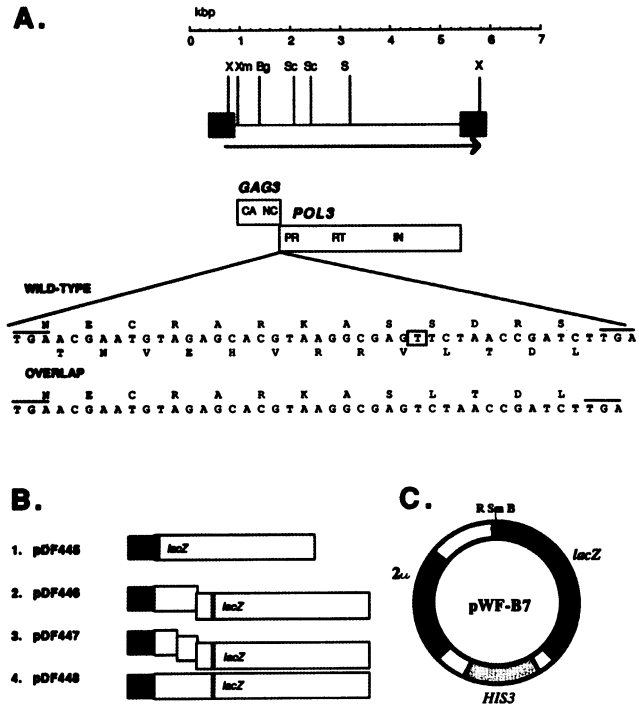


FIG. 1. Ty3 organization and Ty3-*lacZ* fusions. (A) Ty3 organization. A restriction map of Ty3 is shown at the top. Restriction site abbreviations: X, *Xho*I; Xm, *Xmn*I; Bg, *Bgl*II; Sc, *Sca*I; S, *Sal*I. The direction of transcription is shown by the arrow. LTRs (sigma elements) are indicated by solid boxes. The *GAG3* and *POL3* overlapping ORFs are indicated by open boxes. The order of Ty3 protein-coding domains for CA, NC, PR, RT, and IN is indicated. The sequences of nucleotides and encoded proteins within the *GAG3-POL3* overlap region in the WT and the OL mutant are shown. The nucleotide which was deleted in the OL mutant is boxed in the WT sequence. Stop codons in the *POL3* and *GAG3* frames are overlined at the beginnings and ends of the overlapping sequences, respectively. (B) *lacZ* fusions to Ty3 ORFs. *lacZ* fusions were in frame at the *Xmn*I site in *GAG3* or the first *Sca*I site in *POL3*. Ty3 fragments were cloned into *Eco*RI-*Sma*I sites of pWF-B7. (C) High-copy-number yeast shuttle plasmid used for in vivo expression of Ty3-*lacZ* fusions. Restriction sites: *Bam*HI, B; *Eco*RI, R; *Sma*I, Sm.

relative to *GAG3* reading frame expression is comparable to the representation of these proteins in the Ty3 VLP (20).

If *POL3*-encoded proteins are synthesized as Gag3-Pol3, then disruption of the *GAG3* reading frame should block *POL3* expression posttranscriptionally. The Ty3 *GAG3-POL3-lacZ* fusion carried on pDF446 was modified by introduction of 4 bp creating a stop codon at Ty3 nt 1141 to 1143 within the *GAG3* ORF (pDF447). β-gal activity levels in extracts of cells carrying this construct were close to the background level (Table 1). Thus, translation of *POL3* is dependent on translation of at least a portion of *GAG3*, consistent with the existence of a *GAG3-POL3* protein.

Because it was possible that differences in stabilities of fusion mRNAs or in specific activities of β-gal fusions resulted in the different levels of β-gal activity, levels of fusion RNAs and proteins were monitored by using a *lacZ*-specific probe and anti-β-gal antibodies. Figure 2 shows the results of these analyses. Cells containing the *GAG3-lacZ* and *GAG3-POL3-lacZ* fusion constructs showed the expected 3.0-kb (lane 1) and 4.5-kb (lane 2) transcripts, respectively. The *GAG3-lacZ* transcript, however, was present at 1.7-fold the level of the *GAG3-POL3-lacZ* fusion transcript. Because both fusion constructs have the same promoter, this difference in transcript levels could have arisen from differences in mRNA stability. In both cases, several higher-molecular-weight mRNA species were seen which are probably run-on transcripts. Cells containing the *GAG3-POL3-lacZ* fusion (lane 2) showed levels of transcripts similar to levels observed for the *GAG3-POL3* fusion into which a nonsense mutation in *GAG3* was introduced (lane 3). Thus, differences in mRNA levels accounted for a fraction of the difference observed between expression levels of the *GAG3* and *GAG3-POL3* fusion constructs but did not account for the loss of activity when the *GAG3* ORF was disrupted.

TABLE 1. Expression of *lacZ* fused to Ty3 sequences

Plasmid	Description	Fusion site	β-Gal activity (U)[a]
pDF445	*GAG3*	*Xmn*I	73.10
pDF446	*GAG3-POL3*	*Sca*I	3.17
pDF447	*GAG3-POL3* with *GAG3* termination	*Sca*I	0.01
pDF448	*GAG3-POL3* OL mutant	*Sca*I	14.06

[a] Units are given as micromoles of o-nitrophenyl galactoside cleaved per minute per A_{600} unit of cells. Ty3::*lacZ* fusions were carried on high-copy-number yeast 2μm shuttle plasmids that were selected by virtue of the *HIS3* marker. Extracts made from four independent transformants were assayed in triplicate, and averages of 12 values are shown. Units were defined by the following formula: $U = 1{,}000[A_{420} - (1.75A_{550})]/(TVA_{600})$, where A_{420} = absorbance of reaction when stopped, A_{550} = absorbance of reaction when stopped, A_{600} = absorbance of cells used, V = volume of cells used in milliliters, and T = time of the reaction in minutes.

The result of immunoblot analysis of extracts of cells carrying different fusion plasmids with anti-β-gal antiserum is shown in Fig. 2C. The *GAG3-lacZ* fusion construct produced a single species, as expected, close to the size of the full-length β-gal protein marker (Fig. 2C, lane 4). The *GAG3-POL3-lacZ* product consisted of two proteins, one slightly larger than the Gag3–β-gal fusion and one of approximately 160 kDa (Fig. 2C, lane 1). The two species were present at similar levels. The 160-kDa protein was close to the size predicted for a Gag3–Pol3–β-gal fusion protein. The size of the smaller protein was about 130 kDa and thus similar to the size predicted for a Gag3–Pol3–β-gal fusion lacking the Gag3 domain. Extracts of cells expressing a *GAG3-POL3-lacZ* fusion with a disrupted *GAG3* ORF did not contain detectable β-gal, consistent with the results of the β-gal activity assay (Fig. 2C, lane 2). The relative β-gal protein levels observed were qualitatively similar to β-gal activities, suggesting that specific activity did not vary grossly among the various fusion proteins.

We hypothesized that the smaller protein which was expressed from the *GAG3-POL3-lacZ* fusion but which was not made when a stop codon was introduced into *GAG3*, was produced by a posttranscriptional or posttranslational mechanism. If it represented a posttranslational processing product of a larger readthrough protein, as suggested by the effect of introducing a stop codon into the *GAG3* ORF, then levels of the smaller and larger proteins would be expected to increase if a synthetic *GAG3-POL3* fusion were expressed. If, on the other hand, this smaller protein were produced from a processed RNA, then the levels of the smaller protein would not be expected to increase dramatically in response to a single-nucleotide deletion. To investigate which of these models could account for the Ty3 proteins, a synthetic

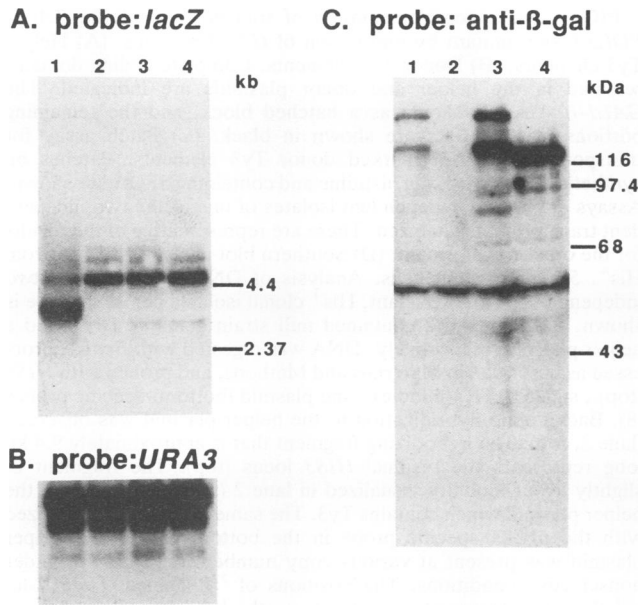


FIG. 2. Expression of Ty3-*lacZ* fusions. (A) Northern blot analysis of Ty3-*lacZ* transcripts in yVB110 transformed with plasmids pDF445 (lane 1), pDF446 (lane 2), pDF447 (lane 3), and pDF448 (lane 4). The positions of relevant size markers are shown to the right of the autoradiogram. The 4.5-kb RNA in lane 1 and fainter bands in all lanes are presumed to be transcripts which were terminated downstream of the major species. (B) Northern blot analysis of *URA3* transcripts. Samples parallel to those shown in panel A, lane 1 to 4, were probed with a *URA3*-specific probe (42). The *URA3*-specific bands in panel B represent plasmid (upper two bands)- and chromosome (lowest band)-derived transcripts. (C) Immunoblot analysis of extracts of cells transformed with pDF446 (lane 1), pDF447 (lane 2), pDF448 (lane 3), and pDF445 (lane 4). The levels of Ty3–β-gal fusion protein were measured by immunoblot analysis of extracts of transformants described in panel A, lanes 1 to 4. The sizes of proteins were extrapolated from those of the protein size markers shown to the right of the autoradiogram. The low-molecular-weight protein was also observed in all lanes, including those containing the protein size markers, and is therefore nonspecific.

GAG3-POL3-lacZ fusion mutant was produced by deletion of nt 1273 within the *GAG3-POL3* overlap region (Fig. 1A), resulting in fusion of the *GAG3* and *POL3* reading frames. The plasmid bearing this mutant Ty3 was designated pDF448. Examination of extracts of cells carrying this plasmid and induced for Ty3 expression showed that levels of this fusion mutant mRNA were about 1.2-fold those of the *GAG3-POL3-lacZ* construct (Fig. 2A, lanes 4 and 2, respectively). Extracts of cells transformed with this mutant and induced with α-factor treatment showed about fivefold the levels of β-gal activity observed in cells transformed with the WT *GAG3-POL3-lacZ* fusion (Table 1). Levels of both the 130- and 160-kDa proteins were 5.3-fold higher in cells containing the *GAG3-POL3-lacZ* fusion mutant than in cells containing the WT *GAG3-POL3-lacZ* construct (Fig. 2C, lanes 3 and 1, respectively), but the ratio of the two species was the same, suggesting that these species were similarly derived from the mutant and WT fusions. This result showed, first, that the apparent mass of the larger Gag3–Pol3–β-gal fusion protein produced from the WT Ty3 sequence is the same as that of the protein initiated in *GAG3* and translated into *POL3* produced when the ORFs are fused. Thus, the natural *POL3*-encoded protein is produced as a fusion protein which includes all or most of the *GAG3*-encoded sequence. Second, this result showed that readthrough of *GAG3* into *POL3* increased the levels of both the large and small proteins expressed from this RNA without significantly changing the level of the RNA; this is consistent with posttranslational production of the smaller protein. The specific derivation of the smaller protein cannot be determined from these results. In Rous sarcoma virus (3) and copia (54), where the protease is encoded in the same ORF as structural protein, expression of downstream sequences is not required for particle formation and processing. The Gag3-Pol3 fusion contains the Ty3 PR domain, and the 130-kDa species is near the size of a β-gal-containing protein predicted if a Gag3–Pol3–β-gal protein is cleaved at the position of the amino terminus of the Ty3 protease (28).

The *GAG3-POL3* fusion polyprotein inefficiently complements transposition of Ty3 donors containing only *cis*-acting sequences required for transposition. The possibility that a stable Gag3–Pol3–β-gal fusion protein was not only synthesized but processed suggested that Gag3-Pol3 might be capable of sponsoring transposition. Cells undergo relatively high levels of Ty3 transposition when elements under control of the *GAL1-10* UAS are induced by growth on galactose-containing medium. The function of a Ty3 mutant in which *GAG3* and *POL3* were fused was measured by using a genetic assay. This assay was similar to that described previously (8). Because *POL3* extends into the downstream LTR, two Ty3 elements are required to provide functions in *cis* and in *trans* for transposition.

Ty3 null strain cells were transformed with two plasmids—one helper and one donor—carrying galactose-inducible Ty3 elements (Fig. 3A and B). The helper plasmid carried bacterial ori and Amp^r functions and yeast *TRP1* and ARS-CEN sequences. The galactose-inducible Ty3 element was either WT or OL. These Ty3 elements were referred to as helper elements because they were a source of *trans*-acting proteins. The donor plasmid was one of four which carried a galactose-inducible Ty3 donor. These plasmids contained the bacterial ori and Amp^r sequences. In addition, they contained yeast 2μm and *URA3* sequences so that they would be maintained in a high copy number and so that cells containing them could be selected for on medium lacking uracil or against on medium containing 5FOA. The yeast *HIS3* gene replaced different amounts of the Ty3 sequence in each donor and was used to monitor the presence of Ty3 (see Materials and Methods). Plasmids pKO253 and pKO254 (36) contained 5 and 26 codons of *GAG3*, respectively. Donor plasmid pJK314 produced an RNA which contained all of the CA coding region but only five codons of NC (29). Donor pJK421 produced RNA which included *GAG3*, CA and NC coding regions, and the beginning of *POL3*, including the PR and RT coding regions. These *HIS3*-marked Ty3 elements were designated donors because they contained *cis*-acting sequences required for replication and transposition (36). Cells transformed with donors alone or helpers and donors were grown on galactose to induce transposition and were replica plated onto YPD to allow loss of the marked donor plasmid. Finally, cells in which transposition occurred were selected by growth on medium lacking histidine and containing 5FOA (Fig. 3C). These colonies typically represent cells which have acquired a genomic copy of the marked Ty3 but no longer contain the donor plasmid.

The ability of the OL helper, compared with that of the WT helper, to complement transposition of each of the donor Ty3 elements was determined. Induction of the WT low-copy-number helper element, together with any of the

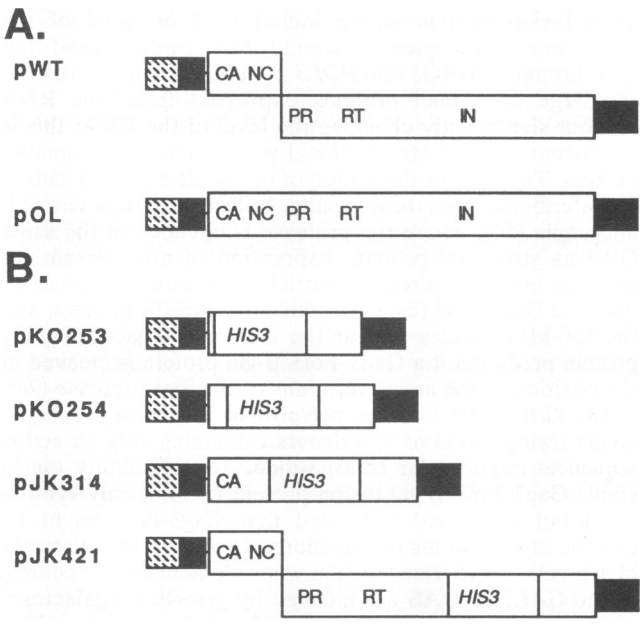
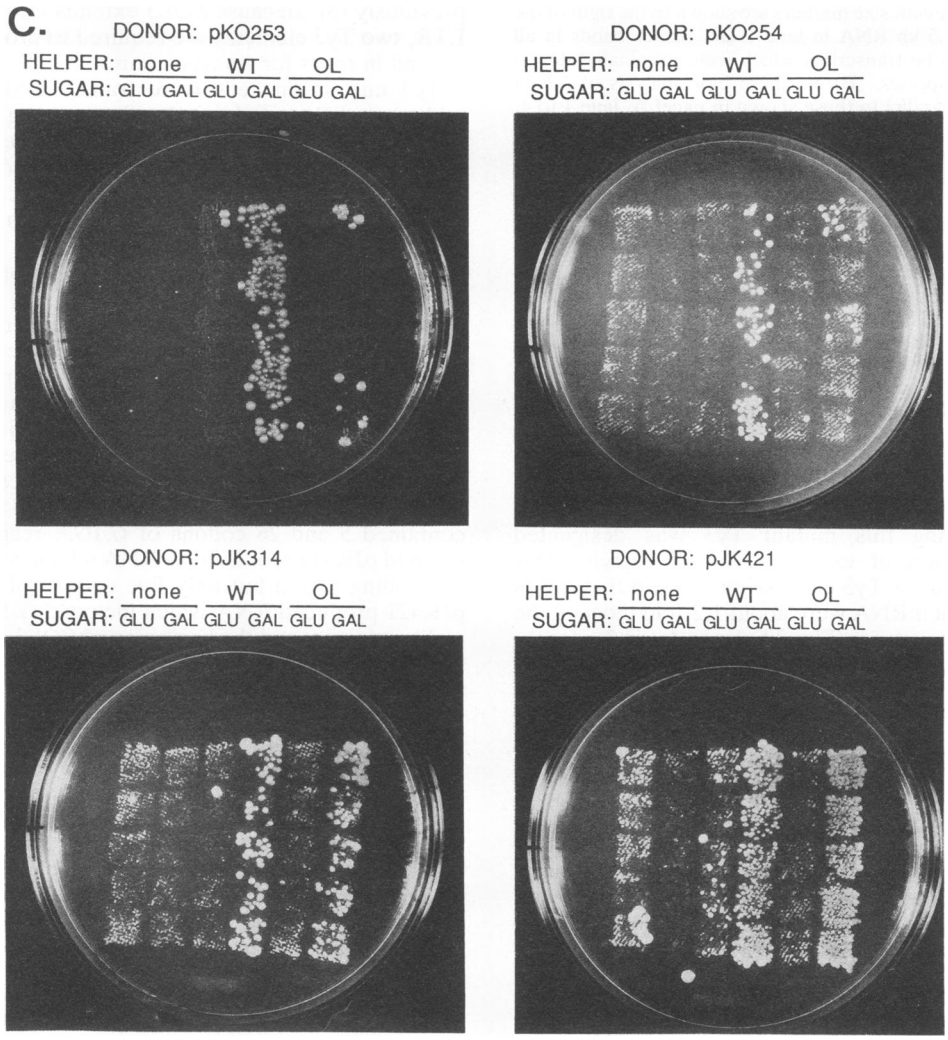


FIG. 3. *trans* complementation of transposition of the *GAG3-POL3* fusion mutant by expression of *GAG3* proteins. (A) Helper Ty3 elements. (B) Donor Ty3 elements. Complete coding domains retained in the helper and donor plasmids are indicated. The *GAL1-10* UAS is shown as a hatched block, and the remaining portions of the LTRs are shown in black. (C) Patch assay for transposition of *HIS3*-marked donor Ty3 elements. Patches on synthetic medium lacking histidine and containing 5FOA are shown. Assays are for five independent isolates of one of the two independent transformants analyzed. These are representative of the results for the other transformant. (D) Southern blot analysis of DNA from His^+, 5FOA-resistant cells. Analysis of DNA samples from two independent, 5FOA-resistant, His^+ clonal isolates per donor type is shown. Lanes 1 and 2 contained null strain genomic DNA and a helper plasmid, respectively. DNA was digested with *Eco*RI, processed as described in Materials and Methods, and probed with *HIS3* (top)-, sigma (LTR) (middle)-, and plasmid (bottom)-specific probes (8). Background hybridization to the helper plasmid was observed (lane 2, top). The hybridizing fragment that is approximately 9.4 kb long represents the residual *HIS3* locus (top). The fragment of slightly lower mobility visualized in lane 2 (middle) represents the helper plasmid which contains Ty3. The same fragment is visualized with the pIBI20-specific probe in the bottom panel. The helper plasmid was present at various copy numbers after growth under nonselective conditions. The positions of ^{32}P-labeled, *Hin*dIII-labeled lambda fragments are shown on the left. Abbreviations and plasmid names are defined in the text.



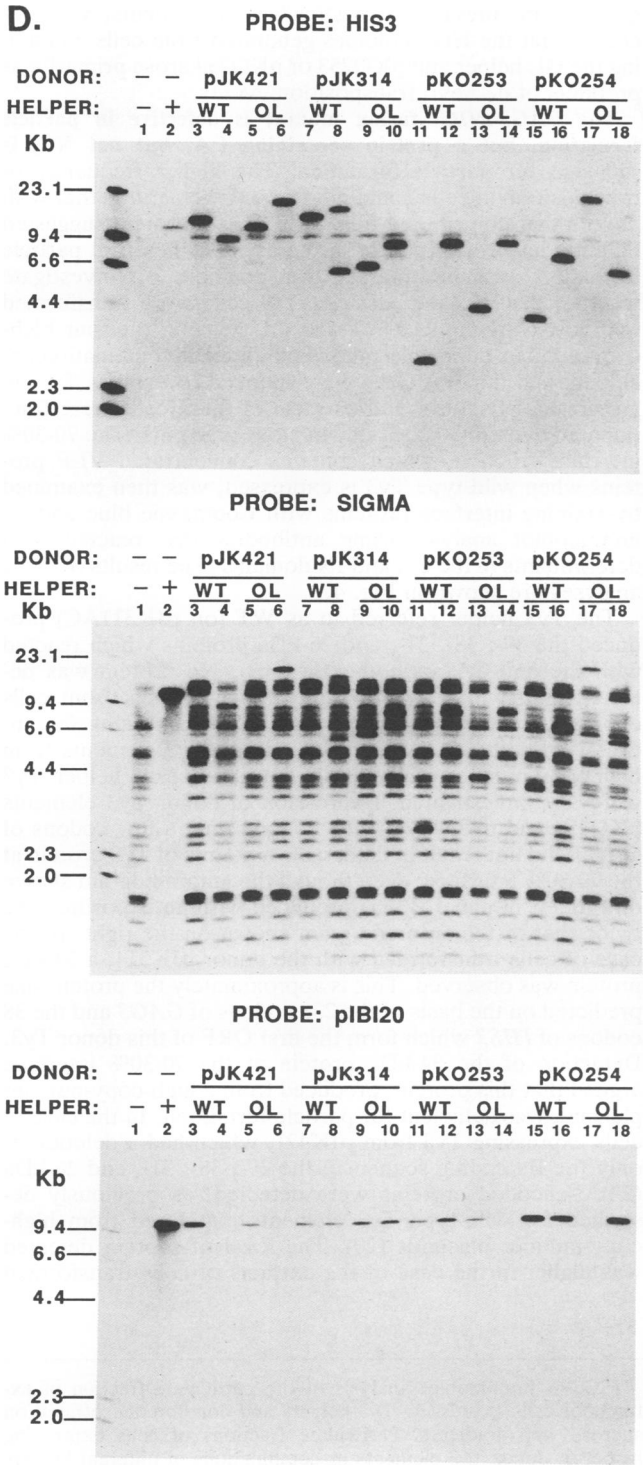
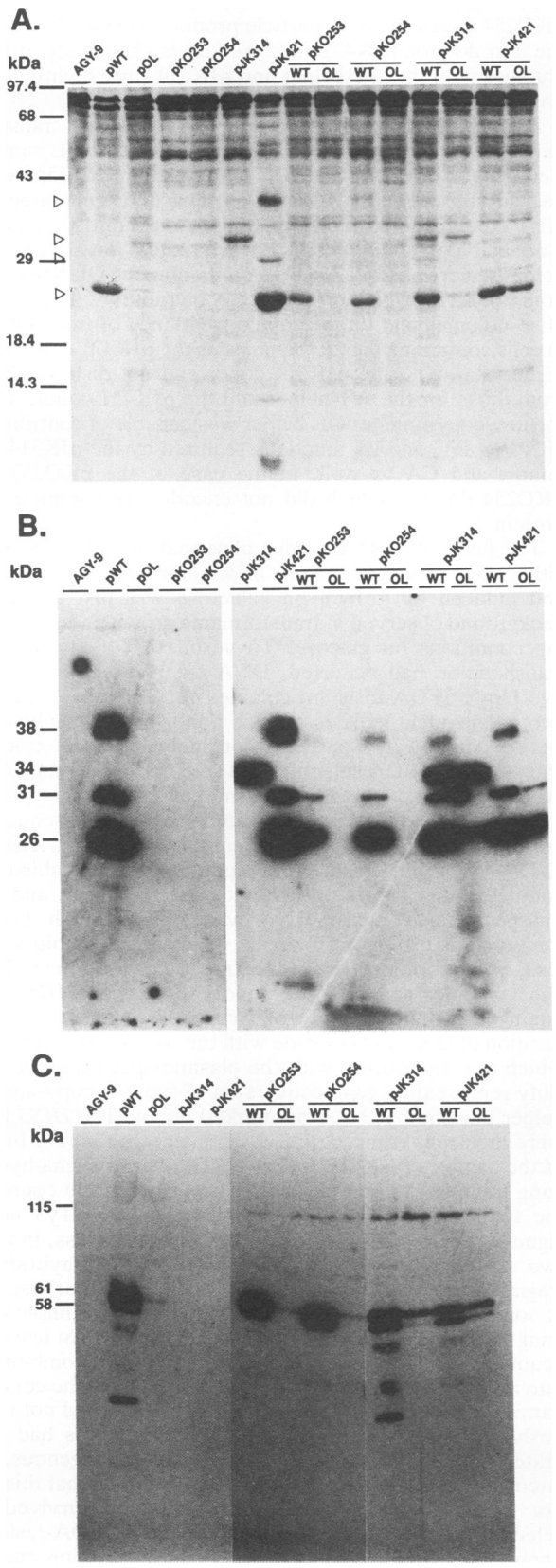


FIG. 3—*Continued.*

four donor elements, resulted in detectable levels of transposition as measured by the patch assay (Fig. 3C). Plasmids pKO253 and pKO254 gave high numbers of His^+ colonies, although pKO253, which contained slightly less of the *GAG3* region than did pKO254, actually produced higher levels of His^+ colonies. The reason for this is not clear, although the lower level of pKO254 transposition correlated with lower levels of wild-type CA protein recovered in the VLP fraction, suggesting that the truncated protein produced from pKO254 interfered with particle production (Fig. 4). Among the four donors, pJK421 gave the highest frequency of His^+ colonies with the WT helper and pJK314 gave slightly lower levels.

Donors which did not encode CA did not transpose efficiently when expressed together with the OL mutant. Cells originally containing donor pKO253 or pKO254, which had an abbreviated *GAG3* region, and pOL generated few His^+ colonies upon replica plating to medium lacking histidine and containing 5FOA. Much higher levels of His^+ colonies were observed in cells containing the OL helper and donor pJK314, which produced CA but not NC. The level of His^+ colonies and the presumed frequency of transposition in cells containing the OL helper plus the pJK421 donor were higher than with the pJK314 donor and not distinguishable from those for the WT helper and the pJK421 donor. Thus, the low-copy-number OL helper was capable of contributing NC, PR, RT, and IN functions required by the pJK314 Ty3 donor, and CA as well, in the case of the pKO253 and pKO254 donors, which did not encode any complete Ty3 protein.

The level of His^+ colonies observed in cells containing either the pKO253 or the pKO254 donor with the OL helper and induced by growth on galactose was just above the background observed in transformants grown under repressing conditions on glucose. To verify that a low level of transposition had occurred, DNA samples extracted from two His^+, 5FOA-resistant colonies generated from each of two independent isolates of each donor-helper combination were examined by Southern blot analysis. These colonies had survived 5FOA selection and so did not have the *URA3* marker carried on the donor plasmid. To determine whether the *HIS3* sequences were associated with plasmid sequences or with a fragment previously containing a sigma element (LTR), as might result from homologous recombination, parallel samples were probed with *HIS3*-, sigma-, and vector-specific sequences. DNA was digested with *Eco*RI, fractionated by electrophoresis, transferred to nitrocellulose, and hybridized (Fig. 3D). In each of the four isolated His^+ colonies analyzed per donor, there was a *HIS3*-containing fragment. In only one case (OL, lane 5) did the position of this band coincide with the position of a fragment which also hybridized with the plasmid-specific probe possibly representing gene conversion of the low-copy-number helper plasmid by the *HIS3*-marked donor. The *HIS3* blots were then rehybridized with a sigma-specific probe. In two of the samples (pJK421 and the WT) a single sigma-hybridizing fragment appeared to have been lost in the course of the transposition, suggesting that insertion of Ty3 into a sigma-containing fragment occurred. Nevertheless, in these two samples, the fact that two different *HIS3*-hybridizing fragments were observed in each lane argued that insertion at some position, in addition to the common sigma-hybridizing fragment, had also occurred, and therefore at least one acquisition of Ty3 had been independent of recombination into the sigma-hybridizing fragment. Because of the complex pattern of LTR hybridization, this experiment did not rigorously exclude the possibility that Ty3 elements had integrated by homologous recombination with endogenous, isolated LTRs. Nevertheless, these results argued that this was not the predominant source of cells which survived the selection. Because acquisition of a His^+, 5FOA-resistant phenotype required induction of Ty3 transcription and did not correlate with integration of plasmid sequences or inte-



gration into previously existing sigma elements, we concluded that the His$^+$ colonies generated from cells containing the OL helper and pKO253 or pKO254 arose primarily as products of de novo transposition events.

The *GAG3-POL3* fusion mutant is defective in particle formation, but a protein containing CA, but not NC, is sufficient for particle formation. The higher frequency of transposition in cells containing the OL helper together with donors that contributed free Gag3 or CA protein suggested that the level of structural proteins, and therefore particle formation, was limiting for transposition. To investigate whether this was the case, AGY-9 cells were transformed with low-copy-number WT and OL helpers, the four high-copy-number donor plasmids, and all eight combinations of helpers and donors. Cells were induced to express Ty3 by growth on galactose, and extracts of these cells were fractionated over 70-30-20% step sucrose gradients. The 70-30% interface fraction, which contains concentrated VLP proteins when wild-type Ty3 is expressed, was then examined by staining interface proteins with Coomassie blue and by immunoblot analysis using antibodies that reacted with determinants in the CA and IN domains. The results of these analyses are shown in Fig. 4.

The Ty3 helper referred to as WT (on pJK311AC) produced the 39-, 38-, 31-, and 26-kDa proteins which reacted with the anti-CA antibody (Fig. 4B). No protein was detected in the interface fraction of the extracts from cells expressing the low-copy-number OL mutant using the anti-CA antibody. Cells expressing donor Ty3 elements from high-copy-number plasmids in the absence of helper Ty3 were also investigated. Expression of donor Ty3 elements pKO253 and pKO254, which contain only a few codons of *GAG3*, did not result in detectable amounts of Ty3 protein at the 70-30% interface, even though the autoradiogram shown on the left in panel B was produced with an exposure time twice that of the autoradiogram shown on the right. In the case of cells transformed with the donor pJK314, a 34-kDa protein was observed. This is approximately the protein size predicted on the basis of the 238 codons of *GAG3* and the 38 codons of *HIS3* which form the first ORF of this donor Ty3. Detection of the 34-kDa protein at the 70-30% interface argued that this protein, produced from a high-copy-number plasmid, was sufficient for particle formation. In the case of cells expressing Ty3 from pJK421, which had a deletion of only the IN coding sequence, the 39-, 38-, 31-, and 26-kDa *GAG3*-encoded proteins were detected, as previously described for wild-type Ty3 elements expressed from high-copy-number plasmids (20). The level of protein detected was higher in the case of the extracts of cells transformed

FIG. 4. Immunoblot analysis of the particulate fraction of extracts of cells expressing Ty3 helpers and donors concentrated on sucrose step gradients. Particulate fractions of cells expressing helper or donor Ty3 elements or combinations of different helpers and donors isolated on sucrose step gradients were examined by SDS-polyacrylamide gel electrophoresis. Extracts were of cells transformed with the plasmids indicated at the tops of the lanes, and 20 μg of protein was analyzed in each lane. (A) Coomassie blue-stained proteins analyzed on an SDS–15% polyacrylamide gel. Immunoblots using anti-CA antibodies (B) and anti-IN antibodies (C) to analyze proteins fractionated on SDS–15% and –10% polyacrylamide gels, respectively, are shown. Protein size markers are indicated on the left in panel A. Open triangles indicate the positions of CA-containing proteins. Sizes of Gag3 and IN species are indicated in panels B and C, respectively.

with donor pJK421 than for cells transformed with the WT helper, as expected for production from a high-copy-number plasmid.

The particle formation defect of the *GAG3-POL3* fusion mutant is rescued by expression together with the CA domain. Particle formation in cells transformed with helpers and donors in combination was also investigated. Expression of pKO253 or pKO254 in cells carrying either pWT or pOL did not result in a qualitative change in the pattern of CA-containing protein, compared with the helper alone. When donor plasmid pJK314, which encoded the 34-kDa protein containing the CA determinant, was expressed together with the WT helper, a pattern which represented a combination of the donor and helper patterns was observed. In this case, the level of the 26-kDa protein was approximately that observed when the helper was expressed alone. It is not possible to deduce from these results whether there was processing of the 34-kDa protein, but the level of the 34-kDa protein suggests that this processing, if it occurred, was not efficient. In the case of pJK314 expressed together with the OL *GAG3-POL3* fusion mutant, a combination which produced wild-type levels of transposition, the 34-kDa protein was present, as with the donor alone, but the 26-kDa protein was also observed. The source of the 26-kDa species was not clear, but on the basis of the fact that only the 34-kDa protein was observed with pJK314 alone, mutant Gag3-Pol3 was at least the source of processing activity to produce the 26-kDa protein. Expression of the donor Ty3 on pJK421, together with either the WT or OL helper, resulted in production of the 39-, 38-, 31-, and 26-kDa species, although these species were present at higher levels with the WT helper than with the OL helper Ty3.

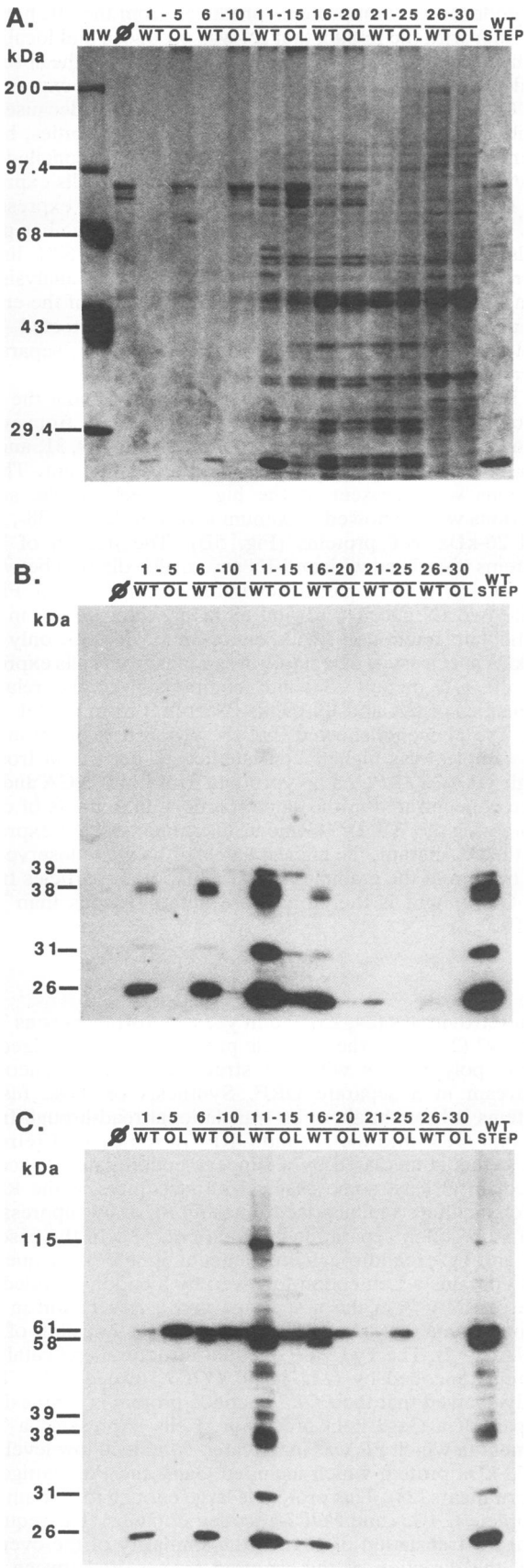
The results of the transposition assay coupled with those from the immunoblot analysis suggested that capsid formation, if it occurred at all, was severely defective for the OL mutant. It was of interest to determine whether catalytic proteins, such as IN, could be detected in the particulate fraction and, in the case of the OL mutant, whether the levels of this protein were affected by the presence of capsid proteins. Aliquots of the same VLP extracts were therefore examined by using the antibody to the IN determinant (Fig. 4C). No donor encoded the antigenic determinant recognized by this antibody. Interestingly, the OL helper produced a reactive protein of 61 kDa. Detection of this protein, but not CA, in cells expressing the OL mutant may reflect greater sensitivity of the immunoblot with the anti-IN antibody. The WT helper produced the 61- and 58-kDa IN species when expressed alone and with each of the four donors. A similar level of IN protein was observed in cells expressing pWT and pJK314, which contributed the 34-kDa protein but not PR, or pWT and the pJK421 donor, which contributed *GAG3* as well as the PR and RT coding sequences. In contrast, the pattern in cells containing the OL helper changed both qualitatively and quantitatively in the presence of donors which contributed *GAG3* protein. Increased levels of the 61-kDa protein and low levels of the 58-kDa protein were observed in cells expressing pOL and pJK314, which produced the 34-kDa protein, or in cells containing the pJK421 donor. Cells expressing pOL and pKO253 or pKO254 displayed the same weak pattern of the 61-kDa protein observed for OL alone. Transposition of pJK421 and pJK314 was complemented to similar levels by WT and OL Ty3 elements. Thus, the level of transposition correlated with the higher levels and increased processing of IN protein.

The possibility that low levels of transposition occurred, dependent solely on proteins produced from the OL helper, together with the finding that IN was produced and localized in the particulate fraction appeared to contradict the absence of detectable capsid protein in extracts of cells expressing the OL mutant on a low-copy-number plasmid. Because the antibodies against CA and IN have different affinities, however, this apparent discrepancy could be reconciled by examination of more concentrated extracts of cells expressing the OL Ty3. Accordingly, extracts of cells expressing OL or WT versions of Ty3 on the high-copy-number plasmids were fractionated in parallel over 10 to 50% linear sucrose gradients and examined by immunoblot analysis by using anti-CA and anti-IN sera (Fig. 5). Assays of the entire linear gradient for OL and WT elements detected Ty3 proteins exclusively in the particulate fractions, separated from most of the cellular proteins.

The particulate Ty3 proteins in cells expressing the OL mutant differed qualitatively and quantitatively from wild-type VLPs. CA antiserum detected proteins of 39, 31, and 26 kDa in extracts of cells expressing the OL mutant. These proteins were present at the highest levels in the same fractions which showed maximum levels of the 39-, 38-, 31-, and 26-kDa WT proteins (Fig. 5B). The pattern of Ty3 proteins visualized with anti-IN serum also differed between the OL and WT samples in several respects (Fig. 5C). First, while two IN species, 61 and 58 kDa, were present in the particulate fractions of cells expressing WT Ty3, only the 61-kDa species was detectable in samples from cells expressing the OL mutant. Second, comparison of the relative intensities of CA and IN bands generated from the OL and WT Ty3 elements showed that the proportion of IN in the OL samples was higher, consistent with derivation from a single OL *GAG3-POL3* polyprotein. Third, while CA and IN were concentrated in the same fractions in extracts of cells expressing the WT Ty3 element, in extracts of cells expressing the OL mutant, the highest levels of IN were observed in the bottom of the gradient. The population of particles from the OL mutant is therefore more heterogeneous than WT VLPs.

DISCUSSION

In retroviruses (24, 25) and in yeast retrotransposons Ty1 and Ty2 (2, 9, 34), the catalytic proteins are synthesized as fusion polyproteins with the structural proteins encoded upstream in a separate ORF. Synthesis of these fusion proteins is brought about by translational readthrough from the first ORF into the second ORF. In the case of retroviruses, this is mediated by a slippery codon pair (26), combined, in at least some cases, with structures in the RNA which facilitate a minus-one frameshift (6) or by suppression of a stop codon separating *gag* and *pol* (53). In the case of Ty1 and Ty2, readthrough is dependent upon a 7-nt sequence that contains a Leu codon followed by a codon decoded by a rare $tRNA^{Arg}$ in the first frame and a Leu codon in the second frame that can be decoded by a plus-one shift of the $tRNA^{Leu}$ (2). The Ty3 VLP contains structural and catalytic proteins encoded by *GAG3* and *POL3*, respectively. This study showed that the *POL3*-encoded protein is synthesized as part of a Gag3-Pol3 precursor. Cells expressing a Ty3 element in which PR was inactivated contained low levels of a 173-kDa protein which included Gag3 and Pol3 antigenic determinants (28). This protein is large enough to contain the complete *GAG3*- and *POL3*-encoded domains. The requirement for translation of *GAG3*, the similarity of the overlap size, the direction of the required frameshift between the



yeast elements, and the fact that Ty3, like Ty1 and Ty2, does not have any of the highly conserved yeast splice sites suggest that the Gag3-Pol3 fusion is produced by frameshifting. In addition, the overlap region has recently been shown to be sufficient to mediate expression of a heterologous fusion protein when inserted in frame between upstream and downstream ORFs, which are plus one relative to each other (16). Together, these results argue that a translational frameshifting mechanism is responsible for production of Gag3-Pol3.

Several retroid elements have now been examined for effects of the expression of fused structural and catalytic polyproteins, in the absence of free structural proteins, on replication and integration. In copia, structural proteins together with protease, are expressed from a spliced RNA. When the splicing reaction was blocked so that only the structural-catalytic precursor polyprotein could be made, neither the precursor protein nor particles were detected (54). In the case of Ty1, a readthrough mutant was shown to produce a detectable fusion protein but was reported to be defective for particle formation (data not shown) (52). Ty3 organization and composition are more closely related to those of the gypsy-like elements and type C retroviruses than to either of these retrotransposons, however, Recently, transposition of Tf1, a gypsy-like element identified in *Schizosaccharomyces pombe*, was demonstrated (31). The proteins of this element required for transposition are encoded by one reading frame, and splicing signals have not been identified (32). Elucidation of the proteins expressed by this element and the nature of the replication complex will be of particular interest in light of the results presented here showing that expression of a *GAG3-POL3* fusion protein is sufficient for low levels of retrotransposition. Retroviral systems have also been examined for the effect of fusing *gag* and *pol* on production of infectious virus. Particles can be produced in Rous sarcoma virus when Gag is fused to cytochrome *c* (48) and Gag–β-gal fusion proteins are incorporated into particles in cells expressing WT viral proteins (27). Nevertheless, fusion of *gag* and *pol* blocked production of infectious Rous sarcoma virus (11, 35), Moloney murine leukemia virus (17), spleen necrosis virus (47), avian leukosis virus (41), and human immunodeficiency virus (37). In Moloney murine leukemia virus, Rous sarcoma virus, and avian leukosis virus, *gag-pol* protein was produced, but neither processing nor particle production was observed. In spleen necrosis virus and human immunodeficiency virus, the polyprotein was produced and processed but particles were not observed. With the exception of human immuno-

FIG. 5. Immunoblot analysis of extracts of cells expressing WT and OL Ty3 elements carried on high-copy-number plasmids fractionated over linear sucrose gradients. Six consecutive sets of five fractions were pooled and concentrated from each linear gradient. The fractions are numbered starting from the bottom of the gradient. Equal amounts of protein were loaded for each pair of WT and OL samples; 15 μg was loaded for all but the bottommost fractions (1 to 5), in which less protein was obtained. The last lane in each panel is a sample from VLP protein fractionated over a sucrose step gradient. (A) Coomassie blue-stained proteins fractionated by SDS-polyacrylamide gel electrophoresis. Sizes of molecular mass markers (lane MW) are indicated to the left. (B) Immunoblot analysis of an identical gel using anti-CA antibody. (C) Immunoblot from panel B reprobed with anti-IN antibody. In panels B and C, sizes of Ty3 proteins are indicated at the left. The null symbol indicates the particulate fraction from cells not transformed with Ty3 plasmids.

deficiency virus, for which complementation testing was not reported, *gag* expression in *trans* complemented formation of infectious virions by the fusion mutants.

Our results differ from those obtained with the retroviral systems in that a low level of particle formation and a low level of transposition were observed in cells expressing only the fusion protein. In addition to the transposition assays described here, the OL helper was expressed in the absence of a donor Ty3 element and insertions were collected in a target plasmid. These transposed Ty3 elements retained the single nucleotide deletion fusing *GAG3* and *POL3* and were flanked by characteristic 5-bp direct repeats, confirming the ability of the OL mutant to transpose at low levels in these cells (29). Although it is clear that transposition of the OL mutant occurred, our experiments do not identify the active particles. It is possible that preferential degradation of the *POL3* domain actually allowed assembly of particles in which *GAG3* protein predominated or that particles with equivalent levels of *GAG3* and *POL3* proteins had low levels of activity. Because retrotransposition occurs intracellularly and does not require budding and infectious particle formation, retrotransposition may have less stringent requirements for particle formation than does the viral life cycle.

Extracts of cells expressing the OL mutant contained the 39-kDa *GAG3* protein but not the 38-kDa species which was present at high levels in cells expressing the WT Ty3. The molar representation of the 38- and 39-kDa species in WT VLPs had previously suggested that they were full-length *GAG3* species derived from the free *GAG3* and *GAG3-POL3* polyproteins, respectively. The amino terminus of PR is encoded 20 codons downstream of the end of *GAG3* (28) and is a candidate for a processing site which would produce a 39-kDa protein from Gag3-Pol3. The finding of the 39-kDa *GAG3* species in the OL mutant particles in this study demonstrated directly its derivation from a *GAG3-POL3* polyprotein.

An unanticipated finding of this study was that the OL mutant was defective primarily in CA. High levels of transposition occurred when a protein containing the CA domain but lacking the NC domain was expressed together with OL mutant Gag3-Pol3. Although mutations in retroviral CA have been shown to disrupt core particle formation (12, 27, 39, 48), that did not exclude a role for NC as a major required structural component of active cores. The experiments presented here showed that CA appears to be the limiting component supplied from free Gag3 and that CA alone was capable of particle formation and association.

Considerable variation in the relative levels of the CA and NC structural domains appears to be tolerated by the Ty3 transposition process, and the requirements for intracellular movement of Ty3 elements are clearly relaxed compared with those for infectious retroviral particle formation. The possibility of relaxed requirements should now be taken into consideration in estimating the potential for intracellular movement of retrotransposons and variants of endogenous retroviruses in the genomes of higher eukaryotic cells.

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