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# Inhibition of Ty1 Transposition by Mating Pheromones in *Saccharomyces cerevisiae*

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**The Ty1 elements in the yeast *Saccharomyces cerevisiae* are a family of retrotransposons which transpose via a process similar to that of retroviral replication. We report here that the Ty1 transposition process can be blocked posttranscriptionally by treatment of cells with mating pheromones. When haploid yeast cells are treated with appropriate mating pheromones, the transposition frequency of a marked Ty1 element driven by the *GAL1* promoter is greatly diminished. Ty1 viruslike particles (VLPs), the putative intermediates for transposition, can be isolated from mating pheromone-treated cells. These VLPs accumulate to normal levels but are aberrant in that they produce very few reverse transcripts of Ty1 RNA both in vivo and in vitro and contain subnormal amounts of p90-TYB and related proteins. In addition, a TYA phosphoprotein product accumulates in treated cells, and some species of TYB proteins have decreased stability. We also show that decreased transposition in mating pheromone-treated cells is not a consequence of simply blocking cell division, since Ty1 transposes at a nearly normal rate in yeast cells arrested in $G_2$ by the drug nocodazole.**

Transposable elements carry out the breakage of host DNA and the insertion of new DNA. Clearly, the frequency of these potentially deleterious reactions must be stringently regulated. In *Escherichia coli*, the well-studied transposon Tn*10* is regulated transcriptionally, translationally, and at the level of the transposition reaction itself (21). In yeast cells, the transposition of Ty1 elements is known to be controlled transcriptionally both by the *MAT* locus and by several *SPT* loci, yet examples of posttranscriptional regulatory mechanisms are few (3).

Ty1 elements, a family of retrotransposons in the yeast *Saccharomyces cerevisiae*, are approximately 6 kb long and consist of a central coding region flanked by long terminal repeats. The two open reading frames in Ty1 include *TYA*, which corresponds to retroviral *gag*, and *TYB*, which corresponds to *pol* (3). Previous studies indicate that Ty1 elements transpose via a mechanism analogous to that of retroviral replication and that the functions and relationships of Ty1-encoded proteins are also very similar to those of retroviral proteins (1, 5, 26, 36). Specifically, TYB proteins are initially synthesized as a fusion product of the *TYA* and *TYB* open reading frames (p190-TYA/TYB). Both TYB and TYA precursor proteins are processed by a Ty1-encoded protease to smaller products (Fig. 1); this processing is essential for transposition (36). It has been shown that TYA proteins are phosphorylated, although it is not known whether the phosphorylation plays any role in transposition (23). Viruslike particles (VLPs) have been isolated from yeast cells in which Ty1 RNAs are overexpressed (19, 24). VLPs are apparently direct intermediates for transposition; they are capable of reverse transcribing Ty1 RNA and integrating Ty1 cDNA, two key steps in the life cycle of Ty1 elements (15, 16, 19, 24).

The conjugation of haploid *MAT***a** and *MAT*α yeast cells requires the actions of peptide mating pheromones. Cells of mating type **a** respond to α-factor secreted by α cells; *MAT*α cells respond to **a**-factor secreted by *MAT***a** cells. Activation of the mating pheromone response pathway leads to cell cycle arrest in $G_1$, changes in cell shape (shmoo formation), cell aggregation, and expression of specific mating-regulated genes (reviewed by Cross et al. [11]).

In this report, we describe a previously unknown phenomenon: Ty1 transposition in haploid yeast cells is blocked by the action of the mating pheromones. The inhibition is posttranslational, since normal amounts of *TYA*-encoded proteins and of some TYB proteins are produced. Upon exposure of yeast cells to mating pheromones, a TYA phosphoprotein accumulates; some forms of TYB proteins are present at very low levels and showed increased instability. The VLPs from mating pheromone-treated cells produce few reverse transcripts of Ty1 RNA both in vivo and in vitro. The possible mechanism and physiological significance of Ty1 transposition regulation by mating pheromone response are also discussed.

## MATERIALS AND METHODS

**Yeast strains and media.** Yeast strain YH49 is derived from YH10 (*MAT***a** *ura3-52 his4-539 lys2-801 GAL*$^+$) by transformation with plasmid pGTy1-H3-*neo* (pJEF1105) (7). YH63 is YH61 (*MAT***a** *ura3-52 his4-539 lys2-801 trp1*Δ*63 GAL*$^+$) carrying plasmid pJEF1724 (pGTy1-H3-*neo* with *TRP1* as the selectable marker for the vector) (3a). YH111 is YH110 [*MAT*α *ura3-167 his3*Δ*200 leu2*Δ*1 sst2*::miniTn*3*(*LEU2*) *GAL*$^+$] carrying pJEF1105. YH110 was constructed by introducing an *sst2* mutation into JB740 using an *sst2*::miniTn*3*(*LEU2*) disruption plasmid (*sst2*::Tn-55). YH60 is YH10 carrying plasmid pGM17, which contains a linker insertion in the protease domain of a pGTy1-H3 element (6a, 36).

Yeast media were prepared as described previously (29). SC/glucose + FOA medium contained 1 mg of 5-fluoroorotic acid (5-FOA) per ml (6). The low-phosphate SC-Met-Cys medium was prepared according the formula of Burkholder (8), with the following modifications: inositol

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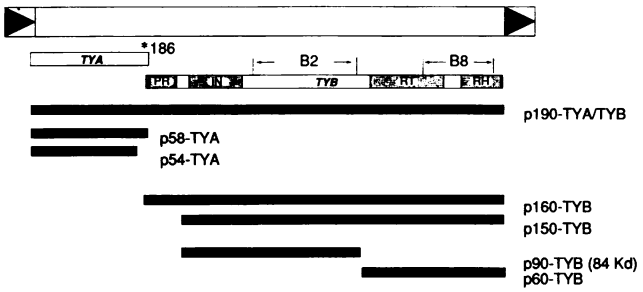


FIG. 1. Ty1 element DNA and protein map. The Ty1 element consists of two long terminal repeat sequences (boxed triangles) surrounding a central coding region (open box). The two long open reading frames, *TYA* and *TYB*, are indicated by open boxes underneath. The domains of *TYB* identified by their homologies to retroviral proteins (14) are indicated by shading and labeled as follows: PR, protease, IN, integrase, RT, reverse transcriptase, RH, RNase H. An approximation of the coding regions for the indicated Ty1-encoded proteins referred to in the text is shown. The positions of the protein sequences used as antigens to generate the antisera discussed in the text are indicated. *, Peptide 186. Arrows indicate extent of sequences used in the form of fusion proteins to generate sera. The sources of the information for the positions of the proteins are as follows: p190-TYA/TYB, p58-TYA, and p90-TYB90 (36); p54-TYA (4, 37); p160-TYB, p150-TYB, p90-TYB, and p60-TYB (37). Certain TYA protein species observed in Ty1 VLPs (p38-TYA and p41-TYA and several less abundant smaller species) reported by Youngren et al. (36) are not observed when the cells are lysed with glass beads rather than zymolyase (15) (see Fig. 2A, 4, and 5). We have not identified any proteins corresponding to the p4, p5, and p6 proteins reported by Adams et al. (1). Kd, Kilodaltons.

was added to 1 mg/liter; $KH_2PO_4$ was replaced by an equal weight of KCl; and $KH_2PO_4$ was added to a final concentration of 30 mg/liter.

**Ty1 transposition assays.** Strain YH49 was used for experiments involving α-factor and nocodazole treatments. The cells were first grown on SC-Ura/glucose plates for 2 days at 30°C. They were then resuspended in YP/2% raffinose medium (identical to YPD except that glucose was replaced by raffinose) (pH 4.0) at $2 \times 10^7$ cells per ml and allowed to grow for 2 to 3 h at 30°C with shaking. At this point, the culture was divided into several 1-ml portions. For α-factor experiments, 5 μl of 1 mM synthetic α-factor (Sigma) dissolved in methanol was added; 5 μl of methanol was added to the control culture. For nocodazole experiments, 10 μl of nocodazole solution (2 mg/ml in dimethyl sulfoxide [DMSO]) was added to the cells; 10 μl of DMSO was added to the control culture. After a 3-h incubation at 30°C, the cells were inspected microscopically for cell cycle arrest induced by exposure to the α-factor or nocodazole. Typically, about 90% of cells were arrested in the treated cultures. Galactose (0.1 ml of 20% [wt/vol] solution) was then added to each culture. The cells were induced for 6 h at 28°C. During this 6-h incubation, additional 1 mM α-factor and methanol (2 μl) were added to the α-factor-treated cells and the control culture, respectively. This was necessary to keep the α-factor-treated cells largely arrested. Finally, the cells were diluted more than 10,000-fold and plated out on SC-Ura/glucose plates at less than 200 cell bodies per plate. Quantitative transposition assays were then carried out essentially as described previously (34). Briefly, single colonies were hand picked to YPD plates and grown overnight at 30°C. Subsequently, the colonies were transferred by replica plating to SC/glucose + FOA plates to select for cells which had lost the plasmid. The colonies grown up on SC/glucose + FOA plates were again transferred by replica plating to YPD containing 650 μg of G418 per ml. The phenotypes of the cells were scored after 1 day at 30°C. Transposition frequency was defined as the percentage of $Ura^-$ ($FOA^r$) colonies which became resistant to G418.

The a-factor experiment was carried out in a similar way with strain YH111. The a-factor solution, obtained by concentrating the medium conditioned by an a-factor-overproducing strain, was the generous gift of Peng Chen and Susan Michaelis. The final concentration of the a-factor used in the experiments was the lowest determined to fully arrest YH111 cells. The control culture was treated with an equal volume of culture supernatant conditioned by a *MATα* strain. The YPD-G418 plates used for this strain contained 65 μg of G418 per ml because the strain is inherently much more sensitive to G418 than is YH49.

The viability of the cells at the end of the 6-h galactose induction was determined as follows. First, the cell density was determined by counting the cells in a Petroff-Hausser bacteria counter under the microscope. The culture was then diluted in water, and 100 to 200 cell bodies were plated out on each YPD plate. The plates were incubated for 2 days at 30°C. The number of the colonies appearing on each plate was determined. The viability of the culture was defined as the ratio of the number of colonies on the YPD plate divided by the total number of cell bodies plated.

For the experiments in which the cells were treated with mating pheromones for a short time, the cells were grown up in SC-ura/glucose and YP/raffinose as described above. Then galactose plus mating pheromone (or galactose plus the solvent as control) were added simultaneously to the culture, and the cells were incubated with shaking at 27°C for 3 h. Subsequently, the cells were diluted and plated out on SC-ura/glucose plates as described above for transposition assays.

**VLP purification and total protein extract preparations.** Ty1 VLPs were purified from 50-ml cultures of galactose-induced YH49 treated with either methanol or α-factor as described above, using a sucrose step gradient (15). The concentrations of the proteins in the VLP fractions were determined by using the Bio-Rad protein assay system.

Total protein extracts for immunoblot analyses were prepared as described previously (35). The relative protein concentrations in these samples were estimated via Coomassie blue staining of the protein gels. Total protein extracts for immunoprecipitation were prepared as follows. YH49 cells were treated with α-factor and induced in galactose for 3 h as described for transposition assays in 1 ml of SC-Met-Cys (for [$^{35}$S]methionine labeling) or low-phosphate SC-Met-Cys (for $^{32}P_i$ labeling and [$^{35}$S]methionine-$^{32}P_i$ double labeling) medium; then 50 μCi of [$^{35}$S]methionine or 250 μCi of $^{32}P_i$ or both radioactive compounds were added to the culture, and the cells were grown in galactose medium for 3 more h before they were harvested. Protein extraction was conducted at 4°C or on ice. The cells were washed once with water and once with PBS (phosphate-buffered saline). They were then resuspended in 50 μl of ice-cold PBS–2 mM phenylmethylsulfonyl fluoride and lysed by vortexing with glass beads. More buffer (100 μl) was added to the lysate, and the mixture was spun at 10,000 rpm for 10 min. The supernatant was transferred to a new tube, and aprotinin (500 U/ml) was added. The extracts were stored on ice overnight before immunoprecipitation. An unlabeled protein extract from an *spt3* strain, which was included in the

immunoprecipitation to decrease background, was prepared similarly except that about 10 times more cells were used.

**Immunological methods.** Antibodies against various Ty1-encoded proteins (Fig. 1) were as follows. Anti-Ty1 VLP sera (hereafter referred to as anti-VLP sera) were raised in rabbits against VLPs purified as described previously (15). These sera recognize all forms of TYA protein that we have tested (3b). Antipeptide 186 was a polyclonal serum raised against a peptide derived from the predicted C-terminal sequence of TYA. Previous results indicated that this serum recognizes only the unprocessed form of TYA protein (p58-TYA) and not the processed form, p54-TYA. This is the basis for the conclusion that there is a protease cleavage site near the C terminus of TYA. Anti-TYB2 polyclonal serum was raised against a TrpE-TYB fusion protein (36) and recognizes the integrase protein p90-TYB. Anti-TyB8 polyclonal serum was raised against a different TrpE-TYB fusion protein and recognizes the reverse transcriptase/RNase H protein. The latter three antibodies were the kind gifts of S. Youngren and D. J. Garfinkel.

Immunoblots were carried out as described previously (30a). Ten percent gels were used for analyzing TYA proteins; in most cases, 7.5% gels were used for analyzing TYB proteins.

Immunoprecipitation of the TYA proteins was performed at 4°C. In tube A, the radiolabeled protein extract was preabsorbed with 50 μl of a 10% *Staphylococcus aureus* cell suspension (Sigma) which had been washed as described previously (20). In tube B, an excess amount of anti-VLP antibodies was incubated with an equal volume of the unlabeled cell lysate from an *spt3* strain, which accumulates no Ty1-encoded proteins (32, 35a). After a 1-h incubation, tube A was spun in a Microfuge centrifuge for 1 min, and the supernatant was mixed with the content of tube B. The mixture was incubated for 1 to 2 h, and another portion (50 μl) of *S. aureus* cells was added. After incubation for at least 1 h, the cells were collected by centrifugation and washed three times in each of the following solutions: (i) PBS–0.1% deoxycholate–1% Triton X-100; and (ii) PBS–0.1% deoxycholate–1% Triton X-100–0.05% sodium dodecyl sulfate (SDS). The final pellet was resuspended in 35 μl of 2× SDS-polyacrylamide gel electrophoresis (PAGE) sample buffer and boiled for 3 min. The sample was clarified by centrifugation for 5 min, and 5 μl of the supernatant was applied to the gel. The TYA proteins labeled with [$^{35}$S]methionine alone were detected by fluorography of the gel, using En$^3$Hance (Du Pont). In the case of the TYA proteins labeled with $^{32}$P or both [$^{35}$S]methionine and $^{32}$P, the gels were directly dried and subjected to autoradiography with an intensifying screen at −80°C. Quantitation of the precipitated TYA proteins was accomplished by cutting out the bands from the dried gel and differentially counting the radioactivity of $^{35}$S and $^{32}$P in a scintillation counter. Correction for $^{32}$P-$^{35}$S spillover in the doubly labeled samples was done by (i) counting bands containing TYA proteins labeled with $^{32}$P alone at the two energy levels to determine the counts due to low-energy $^{32}$P β particles, (ii) subtracting this number of counts from the $^{35}$S counts of the double-labeled samples, and (iii) adding the same number of counts to the $^{32}$P counts of the double-labeled samples.

**Other techniques.** Nucleic acids were extracted from the VLPs and analyzed as described previously (22, 33). Reverse transcriptase assays on the VLPs were performed as described previously (19).

TABLE 1. Inhibition of Ty1 transposition by mating pheromones

| Strain[a] | Treatment | Transposition frequency (%)[b] | Cell viability (%)[c] |
|---|---|---|---|
| YH49 | α-Factor (in methanol) | 2.6 (6/228) | 68 |
| | Methanol | 39.6 (91/230) | 100 |
| YH111 | a-Factor (in conditioned YPD) | <1.2 (0/84) | 16.9 |
| | α-Cell-conditioned YPD | 67.7 (42/62) | 24.4 |
| YH49 | Nocodazole (in DMSO) | 49.4 (129/261) | 38.6 |
| | DMSO | 38.4 (108/281) | 77.7 |

[a] The genotype of YH49 is (*MAT***a** *ura3-52 his4-539 lys2-801 GAL*$^+$); the genotype of YH111 is (*MAT*α *ura3-167 his3Δ200 leu2Δ1 sst2::LEU2 GAL*$^+$). Both strains carry plasmid pGTy1-H3-*neo* (pJEF1105).

[b] Defined as percentage of the FOA-resistant cells that became G418$^r$ after galactose induction and plasmid segregation. Numbers in parentheses are actual numbers observed.

[c] Defined as the ratio of the number of colonies grown up on YPD plates to the number of cell bodies plated to the plates, expressed as a percentage.

## RESULTS

**Reduced transposition of Ty1 elements in haploid yeast cells exposed to mating pheromones.** Plasmid pGTy1-H3-*neo* (pJEF1105), which contains the Ty1-H3 element driven by the *GAL1* promoter (7), was used for all work described in this report. Transposition can be induced by simply switching the carbon source of the media from glucose to galactose. The Ty1 element is marked with the bacterial *neo* gene to facilitate transposition assays. As shown in Table 1, when *MAT***a** cells harboring plasmid pGTy1-H3-*neo* (strain YH49) were treated with α-factor prior to and during galactose induction, the transposition frequency of the *neo*-marked Ty1 element was significantly reduced (there were variations in the degree of inhibition of transposition in different experiments that depended on how well the cells were arrested, but a 10-fold decrease was typically observed).

Previous work suggests that the α- and **a**-factor response pathways are functionally identical (2, 27). Indeed, Ty1 transposition in α cells is also blocked by the action of **a**-factor pheromone (Table 1, strain YH111). In this experiment, it was necessary to introduce an *sst2* mutation into the *MAT*α strain in order to prevent the cells from desensitizing to **a**-factor within the required induction period (9, 10, 12). Treatment of this *sst2* strain with **a**-factor resulted in a block of Ty1 transposition to an undetectable level. To rule out the possibility that Ty1 transposition frequency is decreased in cells responding to mating pheromones simply because these cells are not dividing, we tested the effect of nocodazole, a drug which arrests yeast cells in $G_2$ by destabilizing microtubules, on transposition. The results showed that nocodazole is not inhibitory to transposition (Table 1). Rather, the transposition frequency in nocodazole-treated cells was consistently slightly higher than in the mock-treated control culture in several independent experiments.

In the experiments described above, transposition was assayed after the cells had been exposed to various agents for 9 h. In the case of mating pheromone treatment, this would represent a protracted exposure to pheromone, since zygote formation normally takes only 2 to 3 h. To rule out the possibility that the decreased transposition frequency in these cells might not be physiologically relevant, we also examined Ty1 transposition in cells which had been treated with mating pheromones for a shorter time. In this case, galactose and mating pheromones were added to the cultures simultaneously, and transposition assays were performed 3 h later. The results were identical to those shown in Table 1; i.e., transposition frequency decreased by more than 10-fold

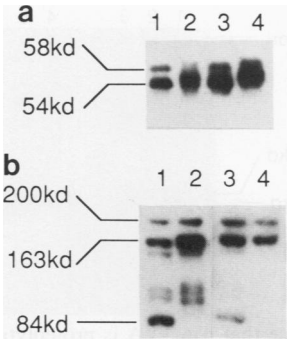


FIG. 2. Immunoblot analyses of Ty1-encoded proteins in total cell extracts. Total protein extracts, prepared from galactose-induced YH49 and YH111 cells which had undergone various treatments, were subjected to immunoblot analyses using the anti-VLP (a) or the anti-TYB2 (b) antibodies. The approximate molecular masses of the major bands as calculated from protein standards run on the same gel are marked at the left in kilodaltons (kd). Lanes: 1, YH49, mock treated; 2, YH49, α-factor treated; 3, YH111, mock treated; 4, YH111, a-factor treated. Approximately 10 μg of total protein was loaded in all lanes.

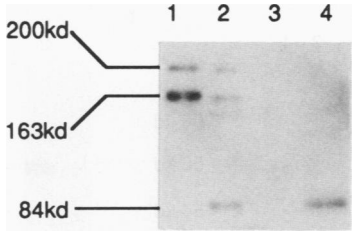


FIG. 3. Instability of certain TYB protein species in α-factor-treated *MAT***a** cells (YH63). Immunoblot analysis was carried out on total protein extracts prepared from α-factor-treated (lanes 1 and 3) or mock-treated (lanes 2 and 4) cells either immediately after galactose induction (lanes 1 and 2) or after the galactose-induced cells were grown overnight in YPD (lanes 3 and 4). Anti-TYB2 antibodies were used. The four cultures were started with equal number of cells; the yields of total protein in all extracts were very similar. Total protein from approximately 5% of each culture was applied to each lane. Therefore, the amounts of TYB protein in the different lanes can be directly compared. The approximate molecular masses of the bands are indicated in kilodaltons (kd).

in the *MAT***a** cells treated with α-factor compared with the mock-treated culture (0.7% versus 7.3%), while a-factor treatment of the *MAT*α cells resulted in a complete inhibition of transposition (<0.4% versus 29%). However, since the level of Ty1 gene products is considerably lower in cells induced for 3 h than in cells induced for 6 h (35a), the longer pheromone treatment and galactose induction condition was used for the subsequent biochemical analyses described below.

**Inhibition of Ty1 transposition by the action of mating pheromones is posttranslational.** To define the stage at which transposition is blocked in mating pheromone-treated cells, we analyzed Ty1 RNA and Ty1-encoded proteins produced in these cells. Northern (RNA) hybridization analysis indicated that the amount and kinetics of mRNA transcribed from *GAL1* promoter-driven Ty1 elements in α-factor-treated cells were normal (data not shown). When *TYA*-encoded proteins from α-factor-treated cells were examined, it was found that they were produced at normal levels (Fig. 2a, lanes 1 and 2). However, these proteins were qualitatively different from those produced in control cells (see Fig. 1 for the map of known Ty1-encoded proteins). The p90-TYB protein or integrase (with an apparent molecular mass of 84 kDa in our gel system), which is the smallest protein recognized by the anti-TYB2 antibodies used, was almost absent in α-factor-treated cells. Instead, the major form of TYB protein detected by this antibody is p160-TYB, thought to be a processing intermediate containing the integrase and reverse transcriptase regions (Fig. 2b, lane 2).

The fact that p160-TYB protein in α-factor-treated cells is less stable than that produced in control (mock-treated) cells was shown by a carbon source pulse-chase experiment. In this experiment, 2-ml cultures of *MAT***a** cells were induced with galactose for 6 h and then split: 1 ml was extracted for protein immediately; the cells in the remaining 1 ml were pelleted and resuspended in 1 ml of glucose medium (YPD) and allowed to grow for 24 h before protein extraction. The state of the Ty1-encoded protein immediately after the pulse and after the chase was then assessed as follows. The entire cultures were harvested, and half of each culture was removed for total protein extraction. Equal volumes of the protein extracts were then subjected to immunoblot analysis. As shown in Fig. 3, by the end of the galactose induction, approximately equal amounts of TYB proteins were produced in the two cultures (lanes 1 and 2). However, after chasing in glucose overnight, the TYB proteins disappeared from the cells which had been exposed to α-factor (lane 3), whereas nearly 100% of the TYB proteins were recovered from the control culture in the fully processed form (p90-TYB).

TYA proteins from treated cells showed abnormal mobilities in gel electrophoresis (this is most obvious in Fig. 4a; compare lanes 2 and 3). The major TYA protein band from treated cells has an apparent molecular mass of about 56 kDa, which is different from those of both the precursor (58 kDa) and the mature (54-kDa) forms of TYA. Antipeptide 186 antiserum (19a), raised against a peptide at the C terminus of the unprocessed TYA protein, fails to recognize this p56-TYA protein, suggesting that it has been proteolytically cleaved (Fig. 5). Furthermore, little if any p56-TYA is observed in Ty1 protease mutants, even in pheromone-treated cells (data not shown).

It has previously been suggested that TYA proteins are phosphorylated (23). We performed immunoprecipitation experiments of metabolically labeled TYA proteins to confirm these results and to determine whether phosphorylation of TYA proteins was altered in *MAT***a** cells treated with α-factor. As shown in Fig. 4a, approximately the same amount of total TYA protein was specifically immunoprecipitated from [$^{35}$S]methionine-labeled cells treated with either methanol or α-factor (lanes 2 and 3). However, the proportion of TYA phosphoprotein with respect to total TYA protein species was increased significantly upon α-factor treatment (Fig. 4b; compare lanes 1 and 2). The cells used for this experiment were doubly labeled with [$^{35}$S]methionine and $^{32}P_i$. The autoradiograph shown in Fig. 4b was performed in such a way that the signals obtained were mostly contributed by $^{32}$P. When the bands were excised from the dried gel and counted in a scintillation counter, the results showed that after correction for the recovery of $^{35}$S counts, the TYA proteins from the α-factor-treated cells contained about six times more $^{32}$P counts than did the control (data not shown).

Ty1-encoded proteins from a-factor-treated *MAT*α cells showed a similar pattern in immunoblots (Fig. 2, lanes 3 and

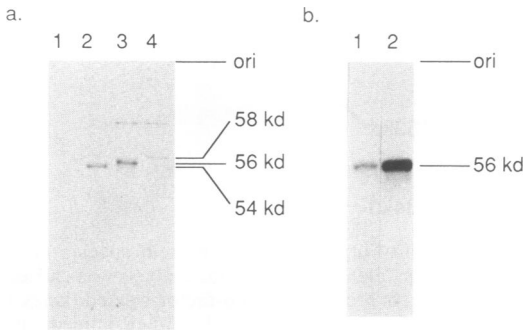


FIG. 4. Immunoprecipitation of metabolically labeled TYA proteins. Total protein extracts were prepared from [$^{35}$S]methionine (a)- or [$^{35}$S]methionine-$^{32}P_i$ (b)-labeled cells which had been treated differently. TYA proteins were then precipitated by using the preimmune serum (lane 1 in panel a) or anti-VLP antibodies (all other lanes) and separated by SDS-PAGE. Fluorography was performed on the gel shown in panel a for 3 days at −80°C. The autoradiograph shown in panel b was performed on the directly dried gel; the exposure was for 2 h at −80°C with an intensifying screen. The estimated molecular masses of the TYA proteins are indicated in kilodaltons (kd). The upper bands in the lanes in panel a were caused by nonspecific absorption of the protein A-containing cells. The difference in appearance of the TYA protein patterns in the immunoprecipitation experiments compared with the results of the immunoblots is due to the fact that the immunoprecipitations were not performed on steady-state-labeled cells but rather on cells which were labeled for 3 h with [$^{35}$S]methionine. Label incorporation is virtually complete within 10 min after label is added, so the proteins studied in the immunoprecipitation experiments are the subpopulation of these proteins that is approximately 3 h old. (a) Lanes: 1, YH49, mock (methanol) treated, preimmune serum precipitated; 2, YH49, mock treated; 3, YH49, α-factor treated; 4, YH60 (carrying protease mutant pGM17), mock treated. (b) Lanes: 1, YH49, mock treated; 2, YH49, α-factor treated.

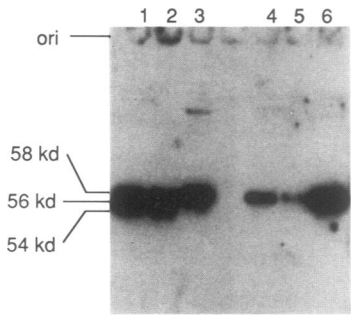


FIG. 5. Evidence that p56-TYA is proteolytically cleaved. Protein extracts from treated and untreated cells were immunoblotted with anti-TYA antibodies that recognize either all of TYA or only the C terminus. Cells were treated with α-factor (lanes 2 and 5) or untreated (other lanes). Cells contained either a wild-type *GAL1*-Ty1 plasmid (lanes 1, 2, 4, and 5) or protease mutant plasmid pGM17. Lanes 1 to 3 were probed with a polyclonal rabbit antibody that was raised against Ty1 VLPs and recognizes all known forms of TYA protein; lanes 4 to 6 were probed with antipeptide polyclonal antiserum 186, which recognizes the C terminus of TYA.

4) except that the defect was more dramatic, possibly because of the *sst2* mutation in the *MAT*α strain.

**VLPs isolated from mating pheromone-treated cells are structurally aberrant and contain a reduced amount of Ty1 reverse transcripts.** All existing evidence suggests that VLPs are intermediates for transposition. VLPs contain TYA proteins, TYB proteins (including reverse transcriptase and integrase), Ty1 RNA, Ty1 DNA, and possibly other as yet unidentified components (1, 15, 19, 24, 26, 36). VLPs were purified from α-factor-treated *MAT***a** cells and analyzed. VLPs were readily detectable at normal levels by assaying for reverse transcriptase activity as well as for TYA protein by immunoblotting (not shown). These VLPs had about 10-fold less reverse transcriptase activity toward endogenous templates and primers ($3.91 \times 10^7$ cpm/mg of VLP protein) than did VLPs isolated from the control culture ($4.83 \times 10^8$ cpm/mg). This correlates well with the level of the transposition defect. However, the VLPs from treated cells showed almost the normal amount of reverse transcriptase activity toward exogenously added poly(rC)/oligo(dG) template/primers ($3.12 \times 10^7$ cpm/mg, versus $6.28 \times 10^7$ cpm/mg for control cells). Southern hybridization analysis of the nucleic acids extracted from these VLPs indicated that little Ty1 DNA was present, suggesting that the VLPs had failed to convert Ty1 RNA into DNA in vivo (Fig. 6b). A smear including many bands, instead of the two discrete species (of 1.6 and 0.6 kb) expected from full-length, double-stranded Ty1 cDNA, was seen in the blot, even in VLPs from mock-treated cells. This is probably due to the relative immaturity of the VLPs owing to the short induction time that had to be used. The VLPs from treated cells apparently contained a lower than normal amount of Ty1 RNA as well (Fig. 6a). We also analyzed the Ty1 proteins contained in the VLPs on immunoblots. As shown in Fig. 7, the molecular masses of the proteins in VLPs were similar to those seen in the total cellular extract (Fig. 2). However, in VLPs from pheromone-treated cells, the p160-TYB protein, detected by anti-TYB2 antibody, was underrepresented (compare Fig. 7b and 2b, lanes 1 and 2). Yet the amount of p60-TYB detected with another antiserum (anti-TYB8; kindly provided by S. Youngren and D. J. Garfinkel) in the same preparation of treated cell VLPs was reduced only slightly

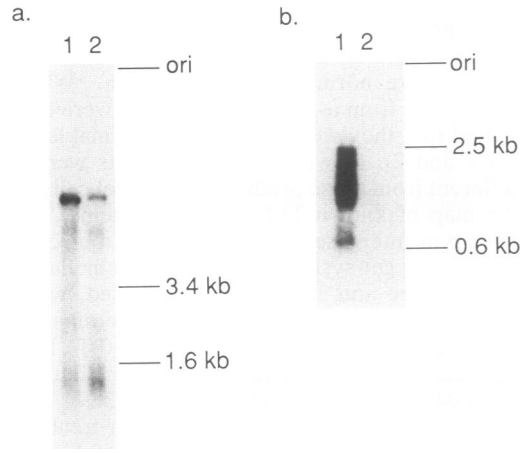


FIG. 6. Analysis of the nucleic acids from VLPs isolated from α-factor- or methanol-treated cells. RNA and DNA were extracted from the purified VLPs and analyzed by Northern (a) and Southern (b) blots, using a $^{32}$P-labeled *neo* probe which hybridized to the marker Ty1 element. Loading was normalized to the amount of total protein present in the VLPs, which corresponds to the total amount of TYA protein in VLP preparations (35a). (a) Lane 1, YH49, mock (methanol) treated; lane 2, α-factor treated. The positions and sizes of the rRNAs are indicated at the right. (b) Lane 1, YH49, mock treated, *Hin*dIII digest; lane 2, YH49, α-factor treated, *Hin*dIII digest. The approximate sizes of the bands are indicated. ori, Origin.

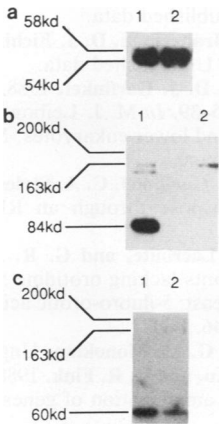


FIG. 7. Analysis of Ty1-encoded proteins in VLPs. Proteins from VLPs isolated from α-factor-treated (lane 2) or mock (methanol)-treated (lane 1) cells were analyzed by immunoblots, using the following antibodies: (a) anti-VLP antibodies which mainly recognize TYA proteins; (b) anti-TYB2 antibodies which detect proteins containing the integrase region; (c) anti-TYB8 antibodies which bind to proteins containing reverse transcriptase. Approximately 10 μg of protein was loaded on each lane. The approximate molecular masses of the major bands are indicated in kilodaltons (kd).

(about twofold) compared with untreated cell VLPs (Fig. 7c). Since different forms of TYB proteins such as p90-TYB and p60-TYB are derived from the same precursor molecule, these results suggest that the stabilities of different species of TYB proteins produced in α-factor-treated cells can vary and that not all TYB proteins are incorporated with equal efficiency into VLPs.

## DISCUSSION

We report here a novel mode of regulation of Ty1 transposition in yeast cells. We have shown that transposition frequency is significantly reduced in response to mating pheromones. This is unlikely to be due simply to a block of cell division since the drug nocodazole, which arrests the cell cycle in $G_2$, is not inhibitory to transposition. Ty1 VLPs isolated from α-factor-treated **a** cells are defective in synthesizing Ty1 DNA both in vivo and in vitro from endogenous template and primer. At the protein level, a TYA phosphoprotein, p56-TYA, accumulates. The accumulation of phosphoprotein p56-TYA might simply reflect hyperphosphorylation of the p54-TYA protein due to an increase in a protein kinase activity. An alternative hypothesis that we cannot absolutely rule out, however, is that TYA processing is somehow aberrant in the treated cells and that the aberrantly processed TYA protein is a better substrate for a protein kinase whose activity does not change upon treatment. In addition, the p90-TYB protein is hardly detectable and certain forms of TYB proteins (p160-TYB) seem to be underrepresented in the VLPs. It remains to be determined which of these biochemical differences between treated and untreated cell VLPs are the direct consequence of mating factor treatment and which are most directly responsible for the transposition defect.

The VLPs purified from **a** cells exposed to α-factor are abnormal in several respects. The major defect is that they contain very little Ty1 cDNA. Consistent with the in vivo data, much less reverse transcriptase activity is detected when exogenous templates and primers are omitted from the in vitro reaction cocktail. However, a nearly normal level of reverse transcriptase activity is detected with use of exogenously added templates and primers. Immunoblot analysis confirmed that these unusual VLPs contain a significant amount of reverse transcriptase protein (p60-TYB). The failure of these VLPs to synthesize Ty1 cDNAs cannot be entirely accounted for by the decreased amount of Ty1 RNA in the treated cell VLPs (Fig. 6b). Since Ty1-encoded proteins are qualitatively different in mating pheromone-treated cells, it is conceivable that the structure of the VLPs is altered in such a way that the template is no longer accessible to the reverse transcriptase. It is also possible that other factors necessary for reverse transcription, e.g., the primer tRNA, may be absent in the VLPs. In addition, there is no detectable p90-TYB (integrase) protein within pheromone-treated cells. Rather, a precursor form of 160 kDa accumulates to higher than normal levels. Within the VLPs, neither of these two species is found. It is paradoxical that relatively normal levels of reverse transcriptase protein are found in the VLPs and yet no integrase protein is found, because these two proteins derive from the same precursor, full-length p190-TYA/TYB protein (Fig. 1). There are several possible explanations for the unusual behavior of TYB proteins in pheromone-treated cells. Any one of these could be the consequence of an aberrant posttranslational modification. (i) The precursor protein may be cleaved prior to assembly into VLPs, and the integrase protein may be aberrantly packaged in pheromone-treated cells; such unpackaged integrase might be very unstable. This seems unlikely because many experiments suggest that retroviral proteases process their substrates after virus particle assembly. (ii) Processing may follow assembly, and processed integrase may leak out of the VLPs or be degraded within them. (iii) Another possibility that cannot be ruled out is a subtle difference in protease cleavage sites in the treated cells; this could result in the selective destabilization of one protein product.

Several components in the mating pheromone signal transduction pathways have been identified. They include the pheromone receptors, the three components of the G protein, and three predicted protein kinases (STE7, STE11, and FUS3) (11, 13, 18, 18a, 25, 30, 31). A simple explanation for the results described here is as follows. Binding of the mating pheromones to their receptors leads to the activation of a protein kinase or kinases through the mating pheromone response pathways; subsequently TYA, and possibly TYB, proteins are hyperphosphorylated by the activated kinase(s); the p90-TYB protein becomes unstable, perhaps as a result of the modification. The resulting abnormal Ty1-encoded proteins form defective VLPs which fail to produce normal amounts of Ty1 reverse transcripts. The lack of Ty1 reverse transcripts results in a decreased transposition frequency. Although the phosphorylated species of TYA proteins comigrate with the major form of the TYA proteins produced in α-factor-treated *MAT***a** cells, we have not proven that the mobility shift seen in TYA proteins from pheromone-treated cells is due solely to phosphorylation. Attempts to dephosphorylate these proteins in vitro with acid phosphatase and alkaline phosphatase were unsuccessful. Therefore, it remains possible that additional types of protein modification occur in response to mating pheromones. As was pointed out above, it is also conceivable, if somewhat less likely, that in treated cells, proteolysis of TYA protein by the Ty1-encoded protease is somehow altered so as to produce a misprocessed form of TYA protein that happens to be a better substrate for the protein kinase. It is also possible that the

decreased stability of certain species of TYB proteins is due to the induction of putative proteases by the mating pheromone response pathway (28).

The mating pheromone response pathway consists of two subpathways; one leads to cell cycle arrest, and one leads to induction of mating-related genes (28). The inhibition of Ty1 transposition may be related to either subpathway. We attempted to distinguish these possibilities by utilizing temperature-sensitive mutant *cdc28* cells (and other *cdc* mutants) arrested in the $G_1$ phase of the cell cycle. Unfortunately, for unknown reasons, only very small amounts of TYB proteins derived from the *GAL1* promoter-driven Ty1 element were produced even when wild-type cells were grown at the nonpermissive temperature (35°C). Currently, few cold-sensitive *cdc* mutants are available. The final resolution of this issue will require the identification and characterization of the factors (such as the proposed kinase[s]) directly responsible for inhibition of transposition.

Previous studies have indicated that the transcription of genomic Ty1 elements is under the control of the mating type; i.e., Ty1 transcription is down-regulated in *MAT*a/α diploid cells compared with mating-proficient haploid cells (17). In the wild, this means that haploid cells have the highest levels of Ty1 mRNA and, presumably, the highest transposition frequency. The work described in this report shows yet another interesting link between Ty1 transposition and the mating system. One can imagine that evolutionarily, it might be advantageous for the cells to limit transposition mostly to the haploid phase, which constitutes only a short part of the yeast life cycle in the wild. The beginning of this gametic phase (meiosis) is also the time during which general recombination activity is at its highest level and new genetic combinations are produced, potentially allowing adaptation to new environments. Thus, it is logical that transposition occurs at a higher frequency in the haploid phase of the sexual cycle. Response to mating pheromones is the prelude to a return to the stable diploid state. While Ty1 elements may be just innocent bystanders of this phenomenon, we speculate that turning off transposition posttranslationally might be one of the processes which returns transposition frequency to a low level and prepares the cell to reenter the genetically stable diploid phase.

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## REFERENCES

1. **Adams, S. E., J. Mellor, K. Gull, R. B. Sim, M. F. Tuite, S. M. Kingsman, and A. J. Kingsman.** 1987. The functions and relationships of Ty-VLP proteins in yeast reflect those of mammalian proteins. Cell **49**:111–119.
2. **Bender, A., and G. F. Sprague.** 1986. Yeast peptide pheromones, a-factor and alpha-factor, activate a common response mechanism in their target cells. Cell **47**:929–937.
3. **Boeke, J. D.** 1989. Transposable elements in *Saccharomyces cerevisiae*, p. 335–374. *In* D. E. Berg and M. M. Howe (ed.), Mobile DNA. American Society for Microbiology, Washington, D.C.

3a. **Boeke, J. D.** Unpublished data.

3b. **Boeke, J. D., L. Braiterman, D. J. Eichinger, S. M. Merbs, and G. M. Monokian.** Unpublished data.

4. **Boeke, J. D., and D. J. Garfinkel.** 1988. Yeast Ty elements as retroviruses, p. 15–39. *In* M. J. Leibowitz and Y. Koltin (ed.), Viruses of fungi and lower eukaryotes. Marcel Dekker Publications, Inc., New York.
5. **Boeke, J. D., D. J. Garfinkel, C. A. Styles, and G. R. Fink.** 1985. Ty elements transpose through an RNA intermediate. Cell **40**:491–500.
6. **Boeke, J. D., F. Lacroute, and G. R. Fink.** 1984. A positive selection for mutants lacking orotidine 5′-phosphate decarboxylase activity in yeast: 5-fluoro-orotic acid resistance. Mol. Gen. Genet. **197**:345–346.

6a. **Boeke, J. D., and G. M. Monokian.** Unpublished data.

7. **Boeke, J. D., H. Xu, and G. R. Fink.** 1988. A general method for the chromosomal amplification of genes in yeast. Science **239**: 280–282.
8. **Burkholder, P. R.** 1943. Vitamin deficiencies in yeasts. Am. J. Bot. **30**:206–211.
9. **Chan, R. K., and C. A. Otte.** 1982. Physiological characterization of *Saccharomyces cerevisiae* mutants supersensitive to G1 arrest by a-factor and alpha-factor pheromones. Mol. Cell. Biol. **2**:21–29.
10. **Chan, R. K., and C. A. Otte.** 1982. Isolation and genetic analysis of *Saccharomyces cerevisiae* mutants supersensitive to G1 arrest by a- and alpha-factor pheromones. Mol. Cell. Biol. **2**:11–20.
11. **Cross, F., L. H. Hartwell, C. Jackson, and J. B. Konopka.** 1988. Conjugation in *Saccharomyces cerevisiae*. Annu. Rev. Cell Biol. **4**:429–457.
12. **Dietzel, C., and J. Kurjan.** 1987. Pheromone regulation and sequence of the *Saccharomyces cerevisiae SST2* gene: a model for desensitization to pheromone. Mol. Cell. Biol. **7**:4169–4177.
13. **Dietzel, C., and J. Kurjan.** 1987. The yeast *SCG1* gene: A $G_{alpha}$-like protein implicated in the a- and alpha-factor response pathway. Cell **50**:1001–1010.
14. **Doolittle, R. F., D. F. Feng, M. S. Johnson, and M. A. McClure.** 1989. Origins and evolutionary relationships of retroviruses. Q. Rev. Biol. **64**:1–30.
15. **Eichinger, D. J., and J. D. Boeke.** 1988. The DNA intermediate in yeast Ty1 element transposition copurifies with virus-like particles: cell-free Ty1 transposition. Cell **54**:955–966.
16. **Eichinger, D. J., and J. D. Boeke.** 1990. A specific terminal structure is required for Ty1 transposition. Genes Dev. **4**:324–330.
17. **Elder, R. T., T. P. St. John, D. T. Stinchcomb, and R. W. Davis.** 1980. Studies on the transposable element Ty1 of yeast. I. RNA homologous to Ty1. Cold Spring Harbor Symp. Quant. Biol. **45**:581–584.
18. **Elion, E. A., P. L. Grisafi, and G. R. Fink.** 1990. *FUS3* encodes a *cdc2*$^+$/*CDC28*-related kinase required for the transition from mitosis into conjugation. Cell **60**:649–664.

18a. **Errede, B.** Personal communication.

19. **Garfinkel, D. J., J. D. Boeke, and G. R. Fink.** 1985. Ty element transposition: reverse transcriptase and virus-like particles. Cell **42**:507–517.

19a. **Garfinkel, D. J., A.-M. Hedge, S. D. Youngren, and T. D. Copeland.** Submitted for publication.

20. **Kessler, S. W.** 1975. Rapid isolation of antigens from cells with a staphylococcal protein A-antibody adsorbent: parameters of the interaction of antibody-antigen complexes with protein A. J. Immunol. **115**:1617–1624.
21. **Kleckner, N.** 1989. Transposon Tn*10*, p. 227–268. *In* D. E. Berg and M. M. Howe (ed.), Mobile DNA. American Society for Microbiology, Washington, D.C.
22. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
23. **Mellor, J., A. M. Fulton, M. J. Dobson, N. A. Roberts, W. Wilson, A. J. Kingsman, and S. M. Kingsman.** 1985. The Ty transposon of *Saccharomyces cerevisiae* determines the synthesis of at least three proteins. Nucleic Acids Res. **13**:6249–6263.

24. **Mellor, J., M. H. Malim, K. Gull, M. F. Tuite, S. McCready, T. Dibbayawan, S. M. Kingsman, and A. J. Kingsman.** 1985. Reverse transcriptase activity and Ty RNA are associated with virus-like particles in yeast. Nature (London) **318**:583–586.
25. **Miyajima, I., M. Nakafuku, N. Nakayama, C. Brenner, A. Miyajima, K. Kaibuchi, K. Arai, Y. Kaziro, and K. Matsumoto.** 1987. *GPA1*, a haploid-specific essential gene, encodes a yeast homolog of mammalian G protein which may be involved in mating factor signal transduction. Cell **50**:1011–1019.
26. **Muller, F., K.-H. Bruhl, K. Freidel, K. V. Kowallik, and M. Ciriacy.** 1987. Processing of Ty1 proteins and formation of Ty1 virus-like particles in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **207**:421–429.
27. **Nakayama, N., A. Miyajima, and K. Arai.** 1987. Common signal transduction system shared by *STE2* and *STE3* in haploid cells of *Saccharomyces cerevisiae*: autocrine cell-cycle arrest results from forced expression of *STE2*. EMBO J. **6**:249–254.
28. **Richardson, H. E., C. Wittenberg, F. Cross, and S. I. Reed.** 1989. An essential G1 function for cyclin-like proteins in yeast. Cell **59**:1127–1133.
29. **Sherman, F., G. R. Fink, and C. W. Lawrence.** 1978. Methods in yeast genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
30. **Teague, M. A., D. T. Chaleff, and B. Errede.** 1986. Nucleotide sequence of the yeast regulatory gene *STE7* predicts a protein homologous to protein kinases. Proc. Natl. Acad. Sci. USA **83**:7371–7375.
30a. **Towbin, H. D., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76**:4350–4354.
31. **Whiteway, M., L. Hougan, D. Dignard, D. J. Thomas, L. Bell, G. C. Saari, F. J. Grant, P. O'Hara, and V. L. MacKay.** 1989. The *STE4* and *STE18* genes of yeast encode potential beta and gamma subunits of the mating factor receptor-coupled G protein. Cell **56**:467–477.
32. **Winston, F., K. J. Durbin, and G. R. Fink.** 1984. The *SPT3* gene is required for normal transciption of Ty elements in S. cerevisiae. Cell **39**:675–682.
33. **Xu, H., and J. D. Boeke.** 1987. High frequency deletion between homologous sequences during retrotransposition of Ty elements in Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. USA **84**:8553–8557.
34. **Xu, H., and J. D. Boeke.** 1990. Localization of sequences required in cis for yeast Ty1 element transposition near the LTR sequences: analysis of mini-Ty1 elements. Mol. Cell. Biol. **10**:2695–2702.
35. **Xu, H., and J. D. Boeke.** 1990. Host genes that influence transposition in yeast: the abundance of a rare tRNA regulates Ty1 transposition frequency. Proc. Natl. Acad. Sci. USA **87**: 8360–8364.
35a. **Xu, H., and J. D. Boeke.** Unpublished data.
36. **Youngren, S. D., J. D. Boeke, N. J. Sanders, and D. J. Garfinkel.** 1988. Functional organization of the retrotransposon Ty from *Saccharomyces cerevisiae*: the Ty protease is required for transposition. Mol. Cell. Biol. **8**:1421–1431.