

Yeast Ty1 Retrotransposition Is Stimulated by a Synergistic Interaction between Mutations in Chromatin Assembly Factor I and Histone Regulatory Proteins

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A screen for host mutations which increase the rate of transposition of Ty1 and Ty2 into a chromosomal target was used to identify factors influencing retroelement transposition. The fortuitous presence of a mutation in the *CAC3* gene in the strain in which this screen was undertaken enabled us to discover that double mutations of *cac3* and *hir3*, but neither of the two single mutations, caused a dramatic increase in the rate of retrotransposition. We further showed that this effect was not due to an increase in the overall level of Ty1 mRNA. Two subtle *cac3* phenotypes, slight methyl methanesulfonate (MMS) sensitivity and reduction of telomeric silencing, were significantly enhanced in the *cac3 hir3* double mutant. In addition, the growth rate of the double mutant was reduced. *HIR3* belongs to a class of *HIR* genes that regulate the transcription of histones, while Cac3p, together with Cac1p and Cac2p, forms chromatin assembly factor I. Other combinations of mutations in *cac* and *hir* genes (*cac3 hir1*, *cac3 hir2*, and *cac2 hir3*) also increase Ty transposition and MMS sensitivity and reduce the growth rate. A model explaining the synergistic interaction between *cac* and *hir* mutations in terms of alterations in chromatin structure is proposed.

Retroviruses are currently under intensive study because they can elicit malignant tumors and cause AIDS. Furthermore, at least 0.1 to 0.6% of the human genome is composed of endogenous retroviruses and long-terminal-repeat (LTR)-containing retrotransposons, which resemble retroviruses in their structural organization and mode of transposition (38). The life cycle of these elements begins with the transcription of an integrated DNA copy of the element and the incorporation of the transcribed RNA into a viruslike particle composed of element-encoded proteins, including the capsid protein, protease, reverse transcriptase, and integrase. The RNA is then reverse transcribed into cDNA, which integrates into a new chromosomal location in the host cell (for a review, see reference 5). Such integration is required for retroviruses to induce disease, for example, by activating a nearby cellular proto-oncogene (70). Likewise, insertions of the yeast Ty1 element (5) can alter the regulation of nearby cellular genes.

Common laboratory yeast (*Saccharomyces cerevisiae*) strains contain five types of Ty elements composed of central regions of DNA flanked by LTRs. Structural proteins and enzymatic activity are encoded within the central region (for reviews, see references 5 and 22). Ty1, Ty2, Ty4, and Ty5 elements are members of the copia class of retrotransposons, while Ty3 elements belong to the gypsy class. Ty1 and Ty2 elements are respectively present at about 30 to 35 and 5 to 15 copies per genome, contain the same LTR sequences (called delta ele-

ments), and have very similar internal regions. The more distantly related retrotransposons Ty3, Ty4, and Ty5 have different sets of LTRs, are present in lower copy numbers, and have not been observed to be insertional mutagens.

Yeast retrotransposons provide an attractive model system in which to define host functions required for retroviral transposition. Mutations that reduce Ty1 and Ty2 transcription levels also reduce transposition (6, 18, 19, 73, 74), since Ty elements transpose through RNA intermediates (3). Transcription of Ty1 and Ty2 elements is also inhibited in mating-incompetent cells (52) and by growth on glycerol rather than glucose (69). Also, both the Ty1 and Ty2 RNA levels and transposition rates are increased by DNA damage (7). In addition, a number of conditions and mutations alter Ty1 transposition via posttranscriptional mechanisms, including translation of Ty-encoded open reading frames (ORFs) (22), reverse transcription of the mRNA into a cDNA copy (10, 11), and growth at 20°C (51, 52).

The ubiquitin-conjugating enzyme Rad6p (Ubc2p) also affects Ty1 transposition. Mutations in *RAD6* increase the overall rate of Ty1 transposition into *CAN1* and *SUP4* without causing an increase in the level of total Ty1 RNA (8, 31, 54). However, when genetically marked Ty1 elements were used, it was determined that the deletion of *RAD6* affected the transposition of some, but not other, elements, and these effects were at the transcriptional level (8). The recent findings that mutations in *RAD6* release transcriptional silencing at telomeres and *HM* loci (28) and that *rad6* mutations enhance the transcription of marked Ty1 elements located in silent rDNA chromatin regions (8) are consistent with the hypothesis that mutations in *RAD6* cause alterations in chromatin structure in certain chromosomal regions, making it easier for Ty1 elements to integrate. Ubiquitination has also been shown to affect Ty3 transposition. Cellular stress, induced by growth at high temperature or in ethanol, inhibits transposition of Ty3

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TABLE 1. Yeast strains used in this study

Strain ^a	Genotype
SL984-6B	<i>MATa ura3-52 his3-Δ200 leu2-1 met8-1 ilv1-1 trp1-901 cac3-1</i>
L1356	<i>MATa ura3-52 his3-Δ200 leu2-1 met8-1 ilv1-1 trp1-901 cac3-1 lys2::his3Δ4</i>
L1561	L1356 <i>hir3-2</i> (also called <i>htr1</i>)
L1562	L1356 <i>rad1::LEU2</i>
L1563	L1356 <i>rad52::URA3</i>
L1635	L1356 <i>rad6::LEU2</i>
L1675	L1356 <i>hir3::LEU2</i>
L1680	L1356 <i>hir1::URA3</i>
L1681	L1356 <i>hir2::URA3</i>
L1685	L1356 <i>cac2::TRP1 hir3::LEU2 [pCAC3]</i>
SL1005-54	<i>MATα his3-Δ200 leu2-1 lys2::his3-Δ4 ade1 cac3-1</i>
SL1006-1B	<i>MATα his3-Δ200 leu2-1 lys2::his3-Δ4 ura3-52 trp1-901 met8-1 cac3-1 hir3-2</i>
SL1006-1D	<i>MATα his3-Δ200 leu2-1 lys2::his3-Δ4 trp1-901 met8-1 cac3-1</i>
SL1006-7B	<i>MATα his3-Δ200 leu2-1 lys2::his3-Δ4 trp1-901 ade1 cac3-1 hir3-2</i>
DC042	<i>MATα ade1 leu2</i>
W303	<i>MATa ura3-1 leu2-3,112 ade2-1 trp1-1 his3-11,15 can1-100</i>
W303Δ3	<i>MATa hir3::HIS3 ura3-1 leu2-3,112 ade2-1 trp1-1 his3-11,15 can1-100</i>
UCC4543	<i>MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 adh4::SUP4-o-TEL^b</i>
L1698	UCC4543 <i>hir1::URA3</i>
L1699	UCC4543 <i>hir2::URA3</i>
L1687	UCC4543 <i>hir3::LEU2</i>
L1678	UCC4543 <i>cac3::hisG-URA3-kan-hisG</i>
L1688	L1678 <i>hir3::LEU2</i>
L1700	UCC4543 <i>cac3::hisG</i>
L1701	L1700 <i>hir1::URA3</i>
L1702	L1700 <i>hir2::URA3</i>
JC364	<i>MATa ura3-167 his3-Δ200 leu2Δ-hisG trp1Δ-hisG Ty588::neo Ty146(tyb::lacZ) Ty1-270his3-AI</i>
L1689	JC364 <i>cac3::hisG-URA3-kan-hisG</i>
L1690	L1689 <i>hir3::LEU2</i>
YJB2306	<i>MATα/MATα ade2-1/ade2-1 can1-100/can1-100 his3-11,15/his3-11,15 leu2-3,112/leu2-3,112 trp1-1/trp1-1 ura3-1/ura3-1</i>

^a DC042, W303, W303Δ3, UCC4543, and JC364 were kindly provided by M. Olson, R. Rothstein, M. A. Osley, D. Gottschling, and D. Garfinkel, respectively.

^b *SUP4-o-TEL*, *SUP4-o* located near telomere VII-L.

without affecting Ty3 transcription. This inhibition can be reversed by overexpression of a protease that cleaves ubiquitin off proteins (43).

In this paper, we describe a genetic screen designed to identify mutations that increase the rate of transposition of Ty1 and/or the closely related Ty2 elements (referred to henceforth together as Ty1 for simplicity). Using this screen, we have uncovered a synthetic interaction between mutations in *CAC3* and *HIR3* that leads to a dramatic increase in Ty1 transposition rates without affecting the overall levels of Ty1 transcription. Simultaneous loss of both genes also increases methyl methanesulfonate (MMS) and UV sensitivities and reduces telomeric silencing and the growth rate. Since *CAC3* encodes a component of chromatin assembly factor I (CAF-I) (34) and *HIR3* controls the levels and balance of histone mRNAs (45, 50, 57), our results suggest that alterations in chromatin structure can increase the efficiency of integration of Ty1 cDNA into the host genome.

MATERIALS AND METHODS

Strains, cultivation conditions, and scoring for markers. Standard yeast cultivation conditions were used (66). Cells were grown on organic complete medium (yeast extract-peptone-dextrose [YPD]) or synthetic complete medium

(SC) lacking nutrients (e.g., uracil [-Ura]). Ura⁻ colonies were selected on medium containing 1 g of 5-fluoro-orotic acid and 12 mg of uracil per liter (+FOA) (4).

Yeast strains used in this study are listed in Table 1. L1356 was made by replacing the *LYS2* locus in SL984-6B (*his3-Δ200*) with a *lys2::his3-Δ4* allele. SL984-6B colonies transformed with pCB1 (carrying *lys2::his3-Δ4* and *URA3*), which had been linearized with *Bst*EII to target integration into the chromosomal *LYS2* locus, were selected on -Ura. Selection for plasmid excision on +FOA medium and subsequent screening for Lys⁻ colonies resulted in the desired transplacement (Fig. 1), which was verified by DNA blot analysis (for details, see reference 9). SL1006 meiotic segregants are from a cross of L1356 with SL1005-54. SL1005-54 is a random spore obtained from a cross of L1356 with DC042.

The *htr1* transposition phenotype was scored by estimating the frequency of His⁺ revertants in independent colonies of approximately equal sizes. Strains were scored for the *hir3* phenotype as described previously (67).

Deletions-disruptions of *RAD1*, *RAD6*, *RAD52*, *CAC2*, *CAC3*, *HIR1*, and *HIR2* were made in L1356 or UCC4543 (Table 1) by using the one-step transplacement method (62) and the appropriate plasmid (described below). Null alleles of *HIR3* were made by a previously described sticky-end PCR method (41), using pZJ31 as a template and primer pairs 170 (CCCCGGCGCGCCCC CCAAATAGACGGTAGCAAGGC)-171 (CTCTTGAAGATGCGAGCCAG) and 172 (CAAGACAAGAGCTACGTGGA)-173 (GGGGGGCGCGCCGGG GCACACTTCTTCCAAAATATG). Sticky extensions (indicated by underlines) carried an *Asc*I site. The annealed fragments were then amplified with primers 171 and 172, and the product was ligated into *Sma*I-digested YIplac128 (*LEU2*) (24) (kindly supplied by R. Gietz) to generate pZJ49. *Asc*I-linearized pZJ49 was then transformed into L1356 to make *hir3* deletions. Yeast transformations were performed by the lithium acetate procedure (29). The *rad1*, *rad6*, and *rad52* deletions were confirmed by complementation tests. All other deletions were confirmed by PCR.

Plasmids. The *URA3*-integrative plasmid pCB1 (9) contains a 1.37-kb *Sma*I-*Bam*HI fragment from pAB100 (63) carrying *his3-Δ4*, which replaces the internal *Hpa*I-*Bam*HI fragment of a *LYS2* gene on the plasmid. The YCp50 (61)-based *CEN-URA3* plasmids pHIR3 (which contains *HIR3*) and pCAC3 (which carries *CAC3*) were partially digested with *Eco*RI or *Hind*III and self-ligated to generate the deletion plasmids diagrammed in Fig. 2B. To generate *CAC3* deletion plasmid pZJ13, a *Bgl*II-*Bam*HI fragment containing *hisG-URA3-hisG* (1) (kindly supplied by E. Alani), cloned into the *Bgl*II site of the *Xba*I-*Sal*I subclone of pCAC3 in Bluescript II KS, was partially digested with *Cla*I and self-ligated.

The following plasmids were used to create deletions-disruptions: pRR46 (58) (kindly supplied by L. Prakash), containing *rad1::LEU2*; pSM22 (kindly supplied by D. Schild), containing *rad52::URA3*; pJJ105, containing *rad6::LEU2* (46) (kindly supplied by L. Prakash); pZJ13, containing *cac3::URA3* (Fig. 2B); pPK104, containing *cac3::hisG-URA3-kan-hisG* (34) (kindly supplied by P. Kaufman); and pHIR1::URA3 and pHIR2::URA3 (68) (kindly supplied by M. A. Osley).

pHIR3-HA, containing a gene with the normal *HIR3* promoter encoding Hir3p tagged with three HA moieties right before the stop codon, was constructed by previously described PCR methods (41, 64). Primer pair 156 (TAT TAGTGGATAAGATCACGAGGGAACAAG [underlined sequence is complementary to that of primer 160]-157 (GTACTTATCTAGAGCGGCCGCAC TG) used the pMPY-3xHA template. The end of the *HIR3* ORF, including the *Bss*HII site (Fig. 2B), was amplified with primers 159 (CAACGACGAAGGTC TTGCAT) and 160 (CGTGATCTTATCCACTAATAG). The annealed fragments were then amplified with primers 159 and 157, and the product was ligated into *Sma*I-digested YEplac195 (24) to generate pZJ53. The *Kpn*I-*Bss*HII fragment in pZJ52 (containing a 10.5-kb *Kpn*I-*Pst*I pHIR3 fragment in YEplac195) was replaced by the *Kpn*I-*Bss*HII piece from pZJ53 to generate pHIR3-HA.

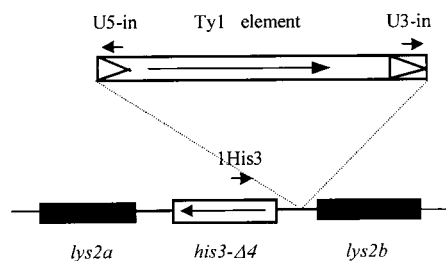


FIG. 1. Ty1 transposition assay. The most common way for the His⁻ strains containing the promoterless *his3-Δ4* allele diagrammed above to revert to His⁺ is by the insertion of an upstream Ty1 element. Such insertions were detected by the appearance of 400- to 500-bp PCR amplicons when the *HIS3* (1His3) and delta element (U5-in) primers were used. No insertions were detected when the *HIS3* (1His3) and delta element (U3-in) primers were used, indicating that the Ty1 elements had to be in the indicated orientation to cause a His⁺ reversion. Arrows in boxes indicate the direction of transcription.

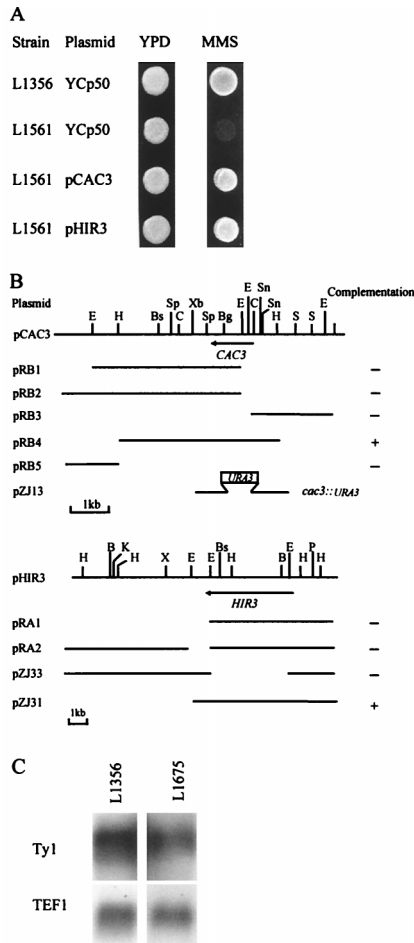


FIG. 2. Characterization of the *htr1* mutant. (A) Complementation of *htr1* MMS sensitivity. Transformants of L1356 (wild type [WT]) and L1561 (*htr1*) with the indicated plasmids (YCp50, pCAC3, and pHIR3) were spotted from $-Ura$ onto YPD and YPD plus 0.02% MMS. (B) Localization of *htr1*-complementing activity. Restriction maps of the cloned fragments in plasmids pCAC3 (top) and pHIR3 (bottom) and subcloned DNA fragments described in the text are shown. +, MMS sensitivity was complemented by the fragment; -, no complementation occurred. Arrows indicate the direction of transcription. Restriction sites are abbreviated as follows: B, *Bam*HI; Bg, *Bgl*II; Bs, *Bss*HII; C, *Cla*I; E, *Eco*RI; H, *Hind*III; K, *Kpn*I; S, *Sal*I; Sn, *Sna*BI; Sp, *Sph*I; P, *Pst*I; X, *Xho*I; and Xb, *Xba*I. (C) Deletion of *HIR3* does not affect levels of Ty1 mRNA. RNA blot analysis of Ty1 (and Ty2) versus *TEF1* (internal control) mRNAs from L1356 and the isogenic *hir3::LEU2* deletion strain, L1675.

Screen for mutants with high rates of Ty1 transposition. L1356 mutagenized with ethyl methanesulfonate to 20 to 40% survival was incubated on YPD at 20°C for 2 weeks. Colonies consisting of approximately 5×10^7 to 1×10^8 cells were spread on $-His$ (or $-His$ containing 0.0125% Casamino Acids so that His^- cells could divide for a few generations). After 3 days at 20°C, which increased the transposition rate to a level more easily measured, the plates were incubated at 30°C for another week and then the number of His^+ revertants per plate was counted.

Transposition rate measurements. Most transposition rates were measured by the *his3-Δ4* assay. In this case, rates of mutation to His^+ were calculated by fluctuation tests, using the equation $P_0 = e^{-N\mu}$ (40), or by the method of the median, using the equation $\mu = f/\ln(N\mu)$ (17). Cells spread on $-Ura$ (to retain plasmids) or YPD were incubated at 20°C until the colonies consisted of about 10^8 cells (on YPD) or 5×10^7 cells (on $-Ura$). For each rate measurement, 10 (or in some cases 20) colonies of equal size were scooped up and each entire colony was spread on a $-His$ plate. Two or three additional colonies of equal size were used to determine cell viability. We used PCR to estimate how many of the His^+ colonies resulted from a Ty1 transposition (Fig. 1). Representative His^+ colonies were analyzed with the *HIS3* primer 1His3 (TGTAATACGCTTTACT AGG) and Ty primers U5-in (ATTGTTGGGATTCCATT) and U3-in (ATAT TATCATATACGGTGTT). Since Ty1 insertions were found in 90% or more of

the His^+ revertants, the His^+ revertant rate was used as an estimate of the Ty1 transposition rate.

Transposition was also assayed as described by Curcio and Garfinkel (14, 15), using a genomic Ty1 element containing the *his3-AI* retrotransposition indicator gene (77). The inversely oriented *his3-AI* gene marking the Ty1 element is inactive because it is disrupted by an intron in the antisense orientation relative to the *his3* gene, but the intron is spliced out during Ty1 transposition, leading to the appearance of a functional *HIS3* gene. Strain JC364 (77) (kindly supplied by D. Garfinkel), which contains the Ty1-270/*his3-AI* transposition reporter, was disrupted first at the *CAC3* gene and then also at the *HIR3* gene, with pPK104 and pZJ49, respectively, to make L1689 and L1690. Strains JC364, L1689, and L1690 were grown to saturation in liquid SC at 20°C. The His^+ frequencies (the number of His^+ prototrophs divided by the total number of colonies) of three independent cultures were averaged to assay the transposition efficiency.

The final Ty1 transposition assay has been described by us previously (39, 54) and involves determining the rate at which normal endogenous Ty1 elements inactivate the *CAN1* gene at 20°C. Rates of mutation to canavanine resistance were calculated by the method of the median, using equation $\mu = f/\ln(N\mu)$ (17), and the fraction of *can1* mutants that contained Ty1 elements was determined by DNA blot analysis.

Analyses of DNA, RNA, and protein. Restriction fragments to be sequenced were subcloned into Bluescript II KS (Stratagene) and sequenced by the dideoxy chain termination method, using Sequenase version 2.0 (U.S. Biochemical Corp.). The *cac3* alleles from L1356 and L1561 were cloned for sequencing by gap repair (48) of *Bss*HII- and *Sna*BI-digested pCAC3.

RNA and DNA blot analyses were performed as described previously (9, 54). The probes used to detect Ty1 and *TEF1* mRNAs were, respectively, a 5.6-kb *Xho*I fragment isolated from pNN166 and an 840-bp *Eco*RI-*Hind*III fragment from pSP36. Western blot analysis was performed on cells transformed with pHIR3-HA or pHIR3, using a 1:1,000 dilution of primary antihemagglutinin (anti-HA) mouse monoclonal antibody (BAbCo, Inc., Berkeley, Calif.).

Indirect immunofluorescence and immunogold labeling. Indirect immunofluorescence was performed as previously described (21), using a 1:5,000 dilution of anti-HA mouse monoclonal antibody, rabbit polyclonal anti-Rap1, and antinuclear pore (BAbCo, Inc.) as well as a fluorescein isothiocyanate-conjugated secondary antibody. Processing and immunogold labeling were performed as described elsewhere (2) with the changes noted below. Cells fixed overnight at 4°C in buffer (4% sucrose in 100 mM sodium phosphate [pH 7.5]) containing 1% acrolein and 4% paraformaldehyde were washed twice with this same buffer for 10 min and then dehydrated at room temperature in an ethylene glycol concentration gradient (25, 50, 75, 90, and 90% for 15, 15, 15, 10, and 10 min, respectively). Infiltration was carried out over a period of 3 days. The primary antibody, HA mouse monoclonal antibody, was used at a 1:1,000 dilution. The secondary antibody was 10-nm gold particles attached to goat anti-mouse antibody (Nanoprobe, Inc., no. CG1001). Grids stained with 1% (wt/vol) uranyl acetate (aqueous) for 10 min and then washed three times for 5 min each in distilled water were stained for 4 min with lead citrate.

RESULTS

An assay for Ty1 transposition. The assay used to determine Ty1 transposition rates was based on the observation that His^+ revertants of a plasmidborne *his3-Δ4* allele that lacks a promoter result either from insertions of Ty1 into the region upstream of *his3-Δ4* or from plasmid rearrangements (3, 63). To eliminate the occurrence of His^+ revertants due to plasmid rearrangements, we made a haploid strain, L1356, that bears a large deletion at the normal *HIS3* locus and an insertion of the *his3-Δ4* allele in its genomic *LYS2* locus (Fig. 1). By using DNA blot and PCR analyses, we found that 38 of 41 independent His^+ revertants derived from L1356 colonies grown at 20°C contained Ty1 insertions in the 5' region of *his3-Δ4*. As expected, all Ty1 elements detected among His^+ revertants were in the orientation which enabled the enhancer sequences in this element to activate the promoterless *his3-Δ4* gene (Fig. 1). Furthermore, as reported for other assay systems (51, 52), the transposition rate in our assay was increased at low temperature (data not shown).

Isolation of mutants with a high Ty1 transposition rate phenotype and characterization of one mutant strain, L1561. To isolate mutations that increase the rate of Ty1 transposition, we screened mutagenized L1356 (*his3-Δ4*) cells for increased levels of His^+ revertants (see Materials and Methods). Colonies which gave rise to more than five His^+ revertants (on average, there was about 0.75 His^+ revertant/colony) were

retested. Of 1,000 colonies screened, 7 candidates repeatedly gave rise to an increased number of His⁺ revertants. Of these seven, two were MMS sensitive and two were temperature sensitive. Each of these four was crossed with SL1005-54, which also contains the *his3-Δ4* transposition assay system. The MMS or temperature sensitivity failed to segregate with the high transposition rate phenotype in three of these crosses.

In the cross involving the fourth mutant strain, designated L1561, the high transposition rate and MMS sensitivity (Mms^s) phenotypes cosegregated. Similar results were obtained when a high transposition rate, Mms^s segregant (SL1006-7B) from this cross was backcrossed with the parental strain, L1356. Although the Mms^s phenotype could not be scored unambiguously in every tetrad, possibly due to the segregation of heterogeneous background genes, MMS sensitivity clearly segregated at a 2:2 ratio in 16 of 18 tetrads examined in the two crosses. Ten of these tetrads were also scored for the high transposition rate phenotype, which clearly segregated 2:2 in nine tetrads and always cosegregated with Mms^s. Thus, it appeared that the segregation of a single Mendelian mutation, which we originally named *htr1* (and renamed *hir3-2* [see below]), was responsible for the Mms^s and high transposition rate phenotypes in the strains produced by these crosses.

The diploid resulting from crossing the L1356 parental strain with a high transposition rate, MMS-sensitive segregant, SL1006-1B, was not sensitive to MMS, indicating that *htr1* (*hir3-2*) is recessive for MMS sensitivity. We could not determine if the mutation was recessive for the high transposition rate phenotype because Ty1 transcription is down-regulated in *MATα*/*MATα* diploid cells (52, 75).

In addition to being MMS sensitive (Fig. 2A), the L1561 mutant was slightly UV sensitive relative to the L1356 parent, but both strains were equally viable following exposure to gamma-ray doses that cause 70 to 85% lethality (data not shown). The rate of Ty1 transposition in L1561 (4.1×10^{-7}) was increased about 60-fold relative to that in the L1356 parental strain (6.4×10^{-9}).

CAC3 and ORF YJR140c were cloned by functional complementation of MMS sensitivity. The L1561 mutant was transformed with a genomic *S. cerevisiae* library made in the *CEN-URA3* vector YCp50 (61). Transformants obtained on $-Ura$ or $-Ura$ supplemented with 0.01% MMS were tested for resistance to 0.025% MMS in YPD, and 21 resistant transformants that regained MMS sensitivity when the plasmid was forced out by growth on $+FOA$ medium were recovered. Plasmid pCAC3, isolated from one of these transformants, was retransformed into L1561, in which it complemented both MMS sensitivity (Fig. 2A) and the high Ty1 transposition rate (see below). Restriction mapping and partial sequencing of the insert in pCAC3 identified a fragment from chromosome II containing six ORFs, including *CAC3*. By deletion analysis, *CAC3* was identified as the complementing gene (Fig. 2B). PCR analysis showed that 16 of the complementing plasmids contained *CAC3*.

The other five complementing plasmids were identical and have been designated pHIR3. Plasmid pHIR3 complements L1561 for MMS sensitivity (Fig. 2A) and for the high transposition rate phenotype (see below). Restriction mapping and partial sequencing of the insert in pHIR3 identified a 14-kb insertion from chromosome X with four ORFs. Deletion analysis showed that *YJR140c* is responsible for the complementing activity (Fig. 2B). *YJR140c* encodes a potential polypeptide of 1,648 amino acids with a predicted molecular mass of 191.7 kDa. A search of databases originally revealed no significant similarity to any known protein. When this project was nearing

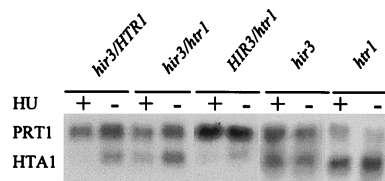


FIG. 3. The *htr1* mutation depressed transcription of *HTA1-HTB1* and failed to complement *hir3*. RNA blot analysis was performed after 30 min of growth in the absence (-) or presence (+) of HU. The diploids were formed by crossing SL1006-1B (*htr1*) and SL1006-1D (*HTR1*) with W303 (*HIR3*) and W303Δ3 (*hir3*). The constitutively transcribed gene *PRT1* was used as an internal loading control since its abundance is not affected by HU.

completion, *YJR140c* was identified as the *HIR3* gene in the *Saccharomyces* genome database (49).

Deletion of *HIR3*, but not *CAC3*, in parental strain L1356 causes MMS sensitivity and a high rate of transposition. L1675, a *hir3::LEU2* deletion mutant created in our original L1356 strain, was MMS sensitive (see Fig. 4) and had an average Ty1 transposition rate of 1.3×10^{-7} , approximately 20-fold higher than the rate in L1356. In contrast, when *CAC3* was disrupted in L1356, the null mutant did not display any increase in MMS sensitivity or Ty1 transposition rate (data not shown).

Since the Ty1 transposition rate is reduced in mutants with lower levels of Ty1 transcription (6, 74), we tested whether the L1675 *hir3* deletion mutant had an altered level of Ty1 mRNA relative to that of the L1356 parental strain. No differences were found when the strains were grown at 30°C, the normal incubation temperature (Fig. 2C), or at 20°C, the incubation temperature used for transposition rate determinations (data not shown).

The *htr1* mutation is an allele of *HIR3*. Since a deletion of *HIR3* reproduced the phenotype of the *htr1* mutation, we suspected that *htr1* was allelic to *HIR3*. This hypothesis was confirmed by genetic complementation. SL1006-1B (*htr1*) was crossed with both W303 (*HIR3*) and W303Δ3 (*hir3::HIS3*) (kindly supplied by M. A. Osley), and SL106-1D was crossed with W303Δ3. The diploids were then examined for the *hir3* phenotype. Mutations in *HIR3* cause cells to lose their ability to repress the transcription of histone *HTA1-HTB1* mRNA in response to the DNA replication-inhibiting drug hydroxyurea (HU) (50). As shown in Fig. 3, *htr1* failed to complement *hir3*, since the *hir3/htr1* diploid (as well as the *htr1* haploid) constitutively expressed *HTA1* in the presence of HU while transcription of *HTA1* was repressed by HU in the *hir3/HTR1* and *HIR3/htr1* diploids. This identified *htr1* as an allele of *hir3*, now called *hir3-2*.

The MMS sensitivity and high Ty1 transposition rate caused by *hir3* are dependent on an additional mutation. Transformation with pCAC3 complemented the MMS sensitivity (Fig. 2A and 4A) and high Ty1 transposition rate (Table 2) phenotypes associated with both the L1561 *hir3-2* mutant strain and strain L1675, which contains a *hir3::LEU2* deletion made in the parental strain L1356. We originally thought that pCAC3 was an extra-copy suppressor of *hir3-2*. However, to our surprise, we found that the L1356 parental strain contained a mutation in *CAC3*. The *CAC3* alleles in L1356 and L1561 were cloned by gap repair and sequenced. The *CAC3* gene cloned from these strains contained, relative to the database sequence, a G-to-C change at position 877 and an A inserted between 877 and 878, resulting in a frameshift causing the loss of about 30% of the *Cac3p*. Additional differences were silent or neutral. The sequence of the PCR-amplified *CAC3* gene from SL1005-54 (used in the original cross with L1561 to score for segregation

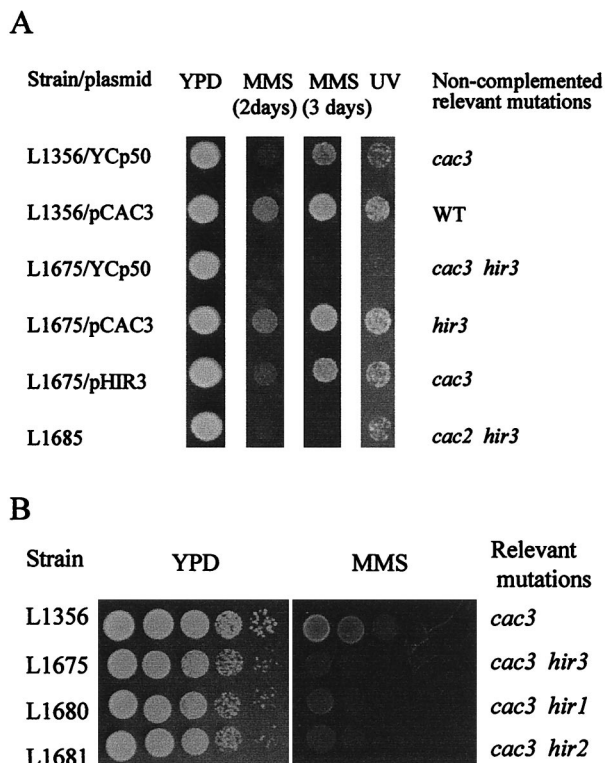


FIG. 4. Double mutations of *hir* and *cac* cause MMS sensitivity. (A) Deletion of *HIR3* causes MMS sensitivity in the presence of *cac3* or *cac2*. L1356 was found to contain a relevant mutation, *cac3-1*. L1356 (*cac3 HIR3*) was transformed with YCp50 or pCAC3, and L1675 (*cac3 hir3*) was transformed with YCp50, pCAC3, or pHIR3. Cells were grown on -Ura medium and were then spotted onto YPD and YPD plus 0.025% MMS. UV irradiation was at 90 J/m². (B) Deletion of *HIR3*, *HIR1*, or *HIR2* in L1356 causes MMS sensitivity. MMS sensitivity was scored by spotting 10-fold serial dilutions of cells on YPD with 0.02% MMS (incubation at 30°C for 2 days).

of *htr1*) indicated the presence of the same frameshift allele, which we have called *cac3-1*.

To distinguish the roles of the *hir3* and *cac3* mutations in causing MMS sensitivity and high Ty1 transposition rates, the isogenic strains L1356 (*cac3-1*), L1561 (*cac3-1 hir3-2*), and L1675 (*cac3-1 hir3::LEU2*) were transformed with pCAC3, pHIR3, or YCp50 (Table 2). The resulting isogenic transformants, mutant for neither *CAC3* nor *HIR3*, either *CAC3* or *HIR3*, or both *CAC3* and *HIR3*, were compared for MMS

sensitivity and Ty1 transposition rates (Fig. 4A; Table 2). The results indicate that the *cac3*, but not the *hir3*, single mutant is slightly sensitive to MMS and that the single *cac3* or *hir3* mutation did not increase the Ty1 transposition rate. In contrast, the double *cac3 hir3* mutant strains were much more sensitive to MMS and had increased Ty1 transposition rates relative to those of wild-type or single-mutant strains.

Mutations in other *HIR* and *CAC* genes also cause MMS sensitivity and high rates of Ty1 transposition when combined with *cac3* and *hir3* mutations, respectively. Since mutations in *HIR1*, *HIR2*, and *HIR3* all have the same effects on the regulation of histone expression (50, 68), we tested whether deletions of *HIR1* and *HIR2* in our parental *cac3-1* strain, L1356, would increase the MMS sensitivity and the Ty1 transposition rate as did mutations in *HIR3*. Indeed, in the *cac3-1* strain, deletion of either *HIR1* or *HIR2* led to an increase in the sensitivity to MMS (Fig. 4B) and caused a dramatic increase in the Ty1 transposition rate (Table 3). Likewise, *cac3 hir1* and *cac3 hir2* double deletions in UCC4543 caused increased sensitivity to MMS, while single *hir* deletions did not (data not shown).

Since Cac3p complexes with Cac1p and Cac2p to form CAF-I (34, 71), we asked whether a *cac2::TRP1* deletion could substitute for the *cac3-1* mutation in causing MMS sensitivity and a high transposition rate in conjunction with a *hir3::LEU2* deletion. Indeed, L1685 (*cac2::TRP1 hir3::LEU2*) is MMS sensitive and has an increased transposition rate (Table 2 and Fig. 4A).

Since the *cac3 hir* double mutants are sensitive to MMS and therefore deficient in DNA repair, we tested the possibility that the increase in Ty1 transposition is a secondary effect of the DNA repair deficiency by deleting a gene of the excision repair pathway (*RAD1*), the error-prone repair pathway (*RAD6*), or the recombination repair pathway (*RAD52*) in L1356 (*cac3-1*). The Ty1 transposition rates in these *rad cac3-1* double mutant strains were determined, and all showed a marginal increase (two- to fourfold) over that of L1356 (Tables 2 and 3).

Simultaneous deletion of *HIR3* and *CAC3* has a dramatic effect on telomeric silencing and growth rate. To examine the effects of mutations in *HIR3* and *CAC3* on telomeric silencing, we used strain UCC4543, which contains the RNA polymerase III (RNAP III)-transcribed gene *SUP4-o* placed close to telomere VII-L (28). Telomeric silencing inhibits the expression of *SUP4-o*, a tyrosyl-tRNA ochre suppressor, thereby causing the loss of suppression of the *ade2-101* ochre marker, leading to red colony color and the absence of growth on -Ade. As shown in Fig. 5A, silencing of *SUP4-o* was reduced in *cac3 hir3*

TABLE 2. Mutations in *hir* and *cac* genes cause a synthetic increase in the rate of Ty1 transposition

Strain/plasmid ^a	Relevant noncomplemented mutation(s)	Transposition rate ^b	Avg. increase (fold) in transposition rate
L1356/pCAC3	Wild type	1.9 (5.0) × 10 ⁻⁸	1.0
L1356/YCp50	<i>cac3-1</i>	4.5 × 10 ⁻⁸ ± 2.4 × 10 ⁻⁸	1.3
L1561/pHIR3	<i>cac3-1</i>	4.0 × 10 ⁻⁸ ± 1.6 × 10 ⁻⁸	1.1
L1675/pHIR3	<i>cac3-1</i>	3.5 (4.3) × 10 ⁻⁸	1.1
L1561/pCAC3	<i>hir3-2</i>	4.8 × 10 ⁻⁸ ± 2.0 × 10 ⁻⁸	1.4
L1675/pCAC3	<i>hir3::LEU2</i>	3.1 (4.9) × 10 ⁻⁸	1.2
L1561/YCp50	<i>cac3-1 hir3-2</i>	6.2 × 10 ⁻⁷ ± 3.0 × 10 ⁻⁷	18.0
L1675/YCp50	<i>cac3-1 hir3::LEU2</i>	3.7 (3.3) × 10 ⁻⁷	10.1
L1685/pCAC3	<i>cac2::TRP1 hir3::LEU2</i>	5.8 (4.5) × 10 ⁻⁷	14.9
L1635/pCAC3	<i>rad6::LEU2</i>	6.0 × 10 ⁻⁸ ± 4.0 × 10 ⁻⁸	1.7

^a All strains are derivatives of L1356, which was found to contain a relevant mutation, *cac3-1*. Strains were transformed with the indicated plasmids.

^b Ty1 transposition rates were determined during growth on -URA in order to maintain selection for the plasmids (see Materials and Methods). When three or more determinations were made, the average and standard deviation are indicated; when two determinations were made, the duplicate value is shown in parentheses.

TABLE 3. Ty1 transposition rates are dramatically increased by *hir1*, *hir2*, or *hir3* mutations in a *cac3-1* strain

Strain ^a	Relevant genotype	Transposition rate ^b	Avg. increase (fold) in transposition rate
L1356	<i>cac3-1</i>	$6.4 \times 10^{-9} \pm 2.4 \times 10^{-9}$	1
L1561	<i>cac3-1 hir3-2</i>	$4.1 \times 10^{-7} \pm 0.6 \times 10^{-7}$	60
L1675	<i>cac3-1 hir3::LEU2</i>	$1.3 \times 10^{-7} \pm .06 \times 10^{-7}$	20
L1681	<i>cac3-1 hir2::URA3</i>	$1.6 (1.6) \times 10^{-7}$	25
L1680	<i>cac3-1 hir1::URA3</i>	$2.0 (2.0) \times 10^{-7}$	30
L1635	<i>cac3-1 rad6::LEU2</i>	$2.0 (1.2) \times 10^{-8}$	2.5
L1562	<i>cac3-1 rad1::LEU2</i>	$2.1 \times 10^{-8} \pm 1.1 \times 10^{-8}$	3.3
L1563	<i>cac3-1 rad52::URA3</i>	$2.8 \times 10^{-8} \pm 1.5 \times 10^{-8}$	4.3

^a All strains are derivatives of L1356.

^b Transposition rates were determined during growth in YPD (see Materials and Methods). When three or more determinations were made, the average and standard deviation are indicated; when two determinations were made, the duplicate value is indicated in parentheses.

double mutants, while deletions of *CAC3* alone reduced silencing to a much lesser extent and deletions of *HIR3* alone had no effect on telomeric silencing. Six, three, and two independent *cac3 hir3*, *cac3*, and *hir3* deletion strains were examined, respectively. Similar data was obtained with single *hir1* or *hir2* and double *hir1 cac3* or *hir2 cac3* deletions (data not shown).

Deletions of *HIR1*, *HIR2*, or *HIR3* in L1356 (*cac3-1*) resulted in a reduced colony size (data not shown). Likewise, *cac3 hir3* double deletions in strain UCC4543 reduced the colony size while single *cac3* or *hir3* deletions in UCC4543 had no detectable effect (Fig. 5B). This colony size difference was easily observed when plates were incubated at 20°C and could also be detected at 30°C during the first 2 days of growth. When grown in liquid YPD, strain UCC4543 had doubling times of 3.5 and 1.8 h at 20 and 30°C, respectively, while the *cac3 hir3* deletion derivative, L1688, had doubling times of 5.4 and 2.5 h at these respective temperatures.

Effects of *cac3 hir3* double mutations on Ty1 transposition at other loci. Above we showed that *cac3 hir3* double mutations increase the rate of Ty1 transposition into *his3-Δ4*, located at the *LYS2* locus. To determine the effect of *cac3 hir3* double mutations on Ty1 transposition into other loci, we used two previously published assays. In the *his3AI* assay, the generation of His⁺ prototrophs is indicative of the transposition of a single marked Ty1 element to any position in the genome (14, 15). Since the majority of transpositions occur in a few genomic hot spots (16, 30), this assay essentially measures the frequency of transposition into these hot spots. In strain JC364 (*CAC3 HIR3*), the frequency (mean ± standard deviation) of His⁺ prototrophs was $5.07 \times 10^{-7} \pm 0.15 \times 10^{-7}$. Deletion of *CAC3* alone did not increase the frequency of His⁺ prototrophs. However, the simultaneous deletion of *CAC3* and *HIR3* (L1690 [*cac3::hisG-URA3-hisG hir3::LEU2*]) resulted in a His⁺ frequency of $2.27 \times 10^{-6} \pm 0.43 \times 10^{-6}$, representing a three- to fivefold increase in transposition rate relative to that of the *CAC3 HIR3* and *cac3 HIR3* parents. Transposition into *CAN1* increased about 10-fold in L1675 (*cac3 hir3*) relative to that in L1356 (*cac3 HIR3*), from 1.0×10^{-7} (duplicate, 1.05×10^{-7}) to 1.15×10^{-6} (duplicate, 9.0×10^{-7}).

Localization of Hir3p. To determine the cellular location of Hir3p, we fused three repeats of the HA epitope tag to the 3' end of the *HIR3* ORF and placed the tagged gene on a multicopy 2-μm plasmid, pHIR3-HA. pHIR3-HA transformants of the MMS-sensitive strain L1675 (*cac3-1 hir3::LEU2*) became resistant to 0.02% MMS (data not shown), demonstrating that the tagged Hir3p (Hir3p-HA) was at least partially functional.

The size of Hir3p-HA, as determined by Western blot analysis, was consistent with the predicted size of 191.7 kDa (data not shown). YJB2306 and L1675 transformed with pHIR3-HA (or pHIR3 as a control) were respectively examined by indirect immunofluorescence microscopy and immunoelectron microscopy, using antibodies specific for the HA epitope. Both techniques localized Hir3p-HA to the nucleus (Fig. 6). About 50 and 10%, respectively, of the YJB2306 and L1675 pHIR3-HA transformants were labeled with the HA antibody, and Hir3p-HA was clearly present in the nucleus, in all of the labeled cells. In contrast, only background immunofluorescence and immunogold labeling were observed in cells transformed with the pHIR3 plasmid and stained with the HA epitope antibody. In double-labeling immunofluorescence experiments with HA antibody and either Rap1p antibody (21) or nuclear pore antibody (MAB414, BAbCo, Inc.), the nuclear Hir3p-HA signal did not colocalize with either Rap1p or the nuclear pores (data not shown).

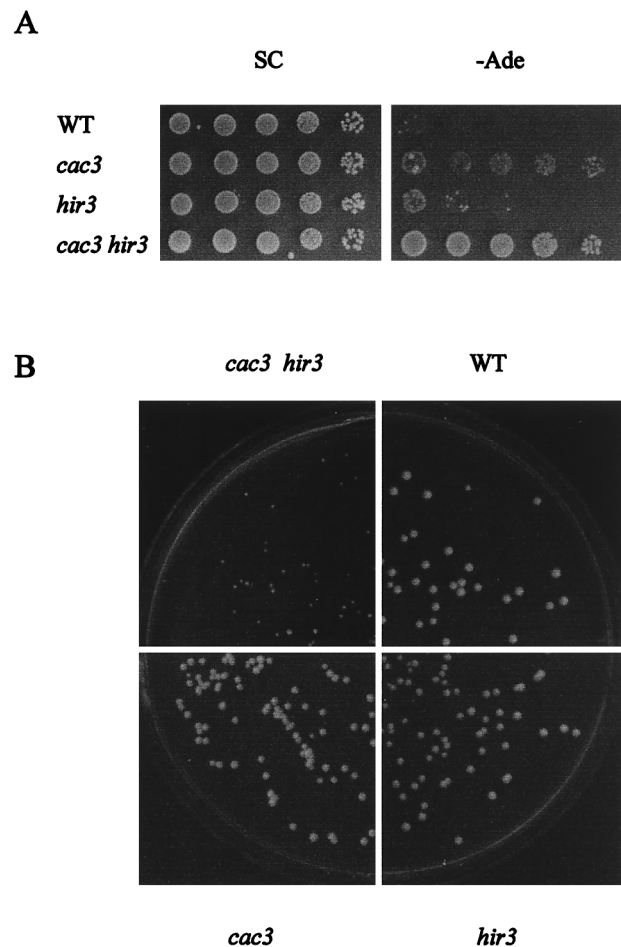


FIG. 5. Silencing and growth defects in *hir3 cac3* double-deletion mutants. Strain UCC4543 (wild type [WT]) and its deletion derivatives L1678 (*cac3*), L1687 (*hir3*), and L1688 (*cac3 hir3*) were used. (A) Silencing of telomere VII-L-located *SUP4-o-TEL* is released in *hir3 cac3* double-deletion mutants. Expression of *SUP4-o-TEL* was assayed by spotting 10-fold serial dilutions on SC (control) and -Ade and incubating the plates at 30°C for 4 days. The slight increase in growth of the *hir3* strain shown on -Ade was not reproducible. (B) Deletion of both *HIR3* and *CAC3* retards cell growth. The indicated strains were diluted, plated on YPD, and incubated at 20°C.

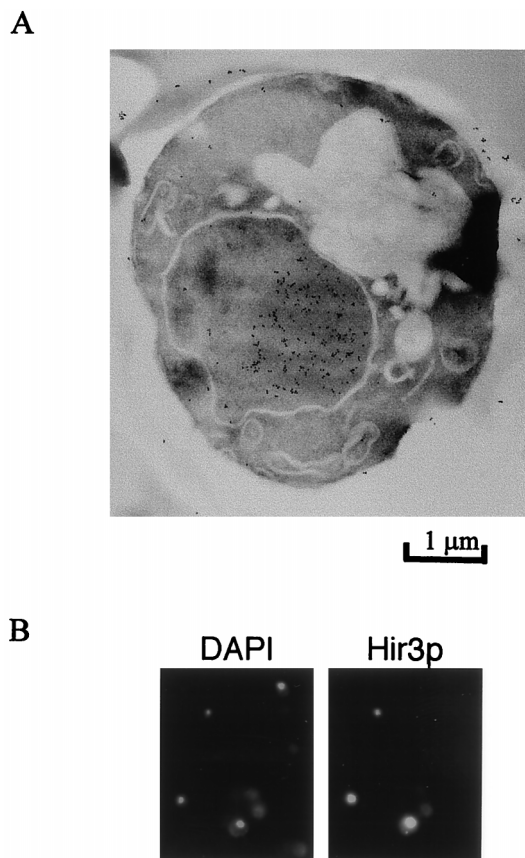


FIG. 6. Nuclear localization of HA-tagged Hir3p. (A) Immunogold labeling of HA-Hir3p in L1675. (B) Indirect immunofluorescence and 4',6-diamidino-2-phenylindole (DAPI) staining of HA-Hir3p in YJB2306.

DISCUSSION

During the analysis of a genetic screen for mutations that increase the rate of transposition of Ty1 into a promoterless *his3-Δ4* target, we discovered that the parental strain in which the search was undertaken contained a frameshift mutation in a gene (*CAC3*) encoding a component of yeast CAF-I (yCAF-I). Since the *cac3* mutant phenotype is very subtle, it is unknown how widespread this mutation is in laboratory stocks. Our search revealed that a newly induced *hir3* mutation in this strain caused a dramatic increase in the Ty1 transposition rate. Mutations in *HIR3* are known to affect the regulation of histone synthesis (45, 50, 68). The observed effect on Ty1 transposition was dependent on the simultaneous presence of both the *cac3* and *hir3* mutations. The two mutations also caused a synthetic increase in growth inhibition as well as in MMS, UV, and cold sensitivity but did not cause gamma-ray sensitivity. The combined presence of mutations in *CAC3* and *HIR3* had no effect on the overall level of Ty1 mRNA, so the increase in the Ty1 transposition rate must result from another mechanism. We propose that this mechanism is an alteration in chromatin structure which makes the DNA more available for Ty1 integration. In support of this hypothesis, silencing of a telomere-proximal RNAP III-transcribed gene was reduced by a combination of *cac3* and *hir3* mutations much more than by the single *cac3* mutation alone. Likewise, in the accompanying paper (32), mutations in *CAC* and *HIR* genes are shown to have a synergistic effect on the release of telomeric silencing of an RNAP II-transcribed gene.

The role of *CAC3* in Ty1 transposition is as a component of CAF-I. yCAF-I (composed of Cac1p, Cac2p, and Cac3p) and human CAF-I (hCAF-I) show a high degree of conservation. Both yCAF-I and hCAF-I preferentially assemble nucleosomes on replicating DNA (33, 34). hCAF-I is thought to be involved in the first step of the nucleosome assembly process, bringing newly synthesized, acetylated isoforms of H3 and H4 tetramers to replicating DNA. Nucleosome assembly is then completed by the addition of H2A-H2B dimers. Mutations in any of the yeast *CAC* genes cause UV but not X-ray sensitivity and a reduction in telomeric silencing (21, 34, 44). Furthermore, the localization of Rap1p, a DNA binding protein which is a significant component of telomeric chromatin, is altered in *cac1* mutants (21). Our finding that a *cac2* deletion (in a *hir3* background) causes increases in Ty1 transposition and MMS sensitivity similar to those observed in the *cac3 hir3* strains suggests that these phenotypes are caused by inactivation of the CAF-I activity. In addition, our finding that mutations in *CAC3* or *CAC2* (synergistically with *hir3*) cause an increase in transposition of Ty1 into a nontelomeric region (*lys2::his3-Δ4* is, respectively, 141 and 58 centimorgans from its telomere and centromere) supports the hypothesis that CAF-I has a general role in chromatin assembly that is not restricted to the formation of heterochromatin.

The findings that deletions of the *CAC* genes are nonlethal and that such deletions do not cause a significant growth defect (34) imply that either replacements for the CAF-I subunits exist or newly replicated DNA can be assembled into chromatin without CAF-I by a parallel pathway. Hat2p has previously been identified to be highly similar to Cac3p (53). A search of the yeast database revealed that Cac3p was 31.3 and 25.1% similar and 23.4 and 18.7% identical to the histone acetylase Hat2p and an uncharacterized ORF, *YMR131C*, respectively, with BLAST (1a) probabilities of 3.0×10^{-30} and 9.0×10^{-18} , respectively. It is possible that Hat2p or Ymr131cp could substitute for Cac3p in the CAF-I complex. The yeast protein most similar to Cac2p is Hir1p (32.3% similarity and 18.2% identity; BLAST probability, 8.0×10^{-17}), and the yeast protein most similar to Hir1p is Hir2p. Cac2p is only about half the size of Hir1p or Hir2p, and the homology between them exists within the N-terminal half of Hir1p. Thus, it is possible that Cac2p and Hir1p have partially overlapping functions.

The effect of mutations in *HIR3* on Ty1 transposition is mimicked by mutations in *HIR1* and *HIR2*. The *HIR1*, *HIR2*, and *HIR3* genes are required for the proper balance of the core histones H2A, H2B, H3, and H4 (45). These histones are encoded by four pairs of divergently transcribed genes, *HTA1-HTB1* and *HTA2-HTB2* (each encoding H2A and H2B) and *HHT1-HHF1* and *HHT2-HHF2* (each encoding H3 and H4). Often the consequence of an imbalance of histones is altered chromatin structure (47), which appears to lead to altered transcription of numerous genes (12, 25, 26) and altered chromosome segregation (42). Hir1p, Hir2p, and Hir3p are required for a feedback control system which autogenously regulates transcription of the *HTA1-HTB1* locus in response to intracellular H2A and H2B levels. Consistent with its role as a transcriptional repressor, Hir2p was previously shown to be located in the nucleus (68). Here we showed that overexpressed Hir3p-HA also localizes to the nucleus. Despite the presence of seven hypothetical transmembrane domains in Hir3p (60), there was no indication from either the immunofluorescence or immunogold labeling studies that Hir3p-HA is associated with a membrane.

The synthesis of histones is coordinated with DNA replication by transcriptional repression of three of the four histone loci, *HTA1-HTB1*, *HHT1-HHF1*, and *HHT2-HHF2*. Derepres-

sion of these loci occurs during late G₁ or early S phase. Mutations in *HIR1*, *HIR2*, and *HIR3* selectively derepress the synthesis of the three histone loci, and such mutations do not appear to be general transcriptional repressors (50, 76).

Mutations in the *HIR* genes suppress the *his4-912δ* and *hys2-128δ* alleles, which are mutant due to the presence of a Ty1 delta sequence in their promoter regions. The *hir* mutations shift the transcription initiation site away from the delta insertion and back to the normal *HIS4* or *LYS2* start site. Yet mutations in the *HIR* genes do not alter the overall levels of Ty1 mRNA (67). Deletion of *HTA1-HTB1* or overexpression of any one of the four histone loci likewise suppresses delta insertion mutations (12). This is consistent with the idea that the *hir* mutations cause an imbalance in the levels of histones and that in a *cac3* background this imbalance enhances the rate of Ty1 transposition.

The dramatic increase in Ty1 transposition associated with *cac3 hir* double mutations is not a property of other DNA repair mutations. Each of the *cac3 hir* double mutants is sensitive to MMS and therefore deficient in DNA repair. It is thus possible that the increase in Ty1 transposition associated with *cac3 hir* mutations is a secondary effect of the DNA repair deficiency and results from unrepaired lesions in the DNA. However, using the promoterless *his3* assay, we found that combinations of *cac3* and deletions of genes from the excision repair pathway (*RAD1*), the error-prone repair pathway (*RAD6*), or the recombination repair pathway (*RAD52*) cause only marginal increases in Ty1 transposition. Since *rad6* and *rad52* mutants are very MMS sensitive, this suggests that the increase in Ty1 transposition caused by the *hir cac3* double mutations is not simply due to unrepaired DNA lesions.

We previously reported that a deletion of *RAD6* dramatically increases the rate of transposition of Ty1 into the *CAN1* gene (54). This is in contrast to our finding, presented here, that deletions of *RAD6* cause only a marginal increase in the transposition of Ty1 into the promoterless *his3* target. To reconcile this discrepancy, we suggest that mutations in *RAD6* affect the chromatin structure of different regions of DNA differently. Indeed, mutations in other genes have been shown to have differential effects on chromatin structure in different loci (47). The *cac3 hir3* double deletions consistently increased the rate of Ty1 transposition when three different assays employing different targets were used.

Other investigators have also examined the effects of various *rad* mutations on the rate of transposition of Ty1 into different targets and obtained different results. Transposition of Ty1 into a plasmidborne copy of the tyrosyl-tRNA *SUP4-o* gene was increased 5- and 20-fold by deletions of *RAD1* and *RAD6*, respectively (31, 36), and appeared to be eliminated by a deletion of *RAD52* (37). In contrast, other investigators found that deletion of *RAD52* did not decrease Ty1 transposition (63, 65).

In addition to its role in duplicating chromosomes, CAF-I has been proposed to be involved in replication-dependent DNA repair mechanisms (34). Indeed, hCAF-I has been shown to participate in excision repair in vitro (23). Our finding that *cac3 hir1*, *cac3 hir2*, *cac3 hir3*, and *cac2 hir3* double mutants are MMS sensitive suggests that CAF-I may also be involved in the repair of MMS damage. It is also possible that an imbalance of histones together with an inactive CAF-I causes an alteration in chromatin structure which makes DNA bases more accessible to UV and MMS damage.

Possible mechanisms for the synergistic effects of *cac* and *hir* mutations on silencing and the rate of Ty1 transposition. Wild-type cells use CAF-I to assemble nascent histones into new nucleosomes in replicated DNA. In *cac* mutant strains, the assembly of new nucleosomes must be accomplished by an-

other pathway. One way to explain the finding that *cac* mutants reduce silencing (21, 34, 44) is to propose that the assembly of new nucleosomes is slower in the absence of CAF-I, allowing time for silencing factors to diffuse away, for acetylases to act on recycled nucleosomes, and for activators to bind to newly replicated DNA (20). Likewise, the synergistic interaction between *cac* and *hir* mutations could be explained if the nucleosome assembly activity substituting for CAF-I in *cac* mutants were further slowed by the *hir* mutations (possibly due to an imbalance in histones) while the bona fide CAF-I activity in *CAC* strains was relatively resistant to the effects of the *hir* mutations. A longer delay in chromatin reassembly in *cac hir* strains would increase the chance that dissociated proteins diffuse away and that recycled histones are modified. After several generations, the result would be a steady-state structure in *cac hir* strains that differed from that in *CAC HIR* strains in both histone modification levels and DNA binding proteins. In silent regions, this could cause a reduction of silencing; in other regions of the genome, it could result in increased availability for Ty transposition.

The increased transposition rate in *cac hir* strains could result from histone modifications and DNA binding proteins that make the DNA structure more available for transposition. Indeed, in vitro experiments suggest that human immunodeficiency virus integration is enhanced by nucleosome-promoted distortions in the DNA double helix which make the major groove more accessible than it is in naked DNA (55, 56). Previous results are consistent with the idea that the conformation of chromatin can affect the integration of Ty1 elements. Mutations in *RAD6* which affect silencing (8, 28) also dramatically reduce the bias for Ty1 integrations to occur preferentially in the promoter rather than in the coding regions of a variety of genes (27, 31, 39, 72). Deletion of *HTA1-HTB1* relaxes (59) the normally strong orientation bias of Ty1 elements that transpose into the promoter region of *CAN1* (72).

Hot spots for integration of various Ty elements depend on host protein complexes assembled on the DNA. Ty5 elements are preferentially targeted for integration by the protein complex assembled at silenced regions (78). Ty3 integration requires binding of the transcription factors TFIIB and TFIIC but is inhibited by RNAP III, suggesting that the Ty3 integration machinery competes with RNAP III for interaction with TFIIB and TFIIC (13, 35). Likewise, Ty1 has also been shown to have a strong preference for integration into regions upstream of genes transcribed by RNAP III (16). We suggest that the combination of *cac* and *hir* mutations may enhance the rate of integration of Ty1 into the promoterless *his3* gene used in this study by creating conditions under which proteins that encourage Ty1 integration bind to the *his3* upstream region.

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