Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 5629–5634, May 1996
Cell Biology

Cellular stress inhibits transposition of the yeast retrovirus-like element Ty3 by a ubiquitin-dependent block of virus-like particle formation

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Communicated by Maynard V. Olson, University of Washington, Seattle, WA, January 30, 1996 (received for review June 22, 1995)

ABSTRACT **Many stress proteins and their cognates function as molecular chaperones or as components of proteolytic systems. Viral infection can stimulate synthesis of stress proteins and particular associations of viral and stress proteins have been documented. However, demonstrations of functions for stress proteins in viral life cycles are few. We have initiated an investigation of the roles of stress proteins in eukaryotic viral life cycles using as a model the Ty3 retrovirus-like element of *Saccharomyces cerevisiae*. During stress, Ty3 transposition is inhibited; Ty3 DNA is not synthesized and, although precursor proteins are detected, mature Ty3 proteins and virus-like particles (VLPs) do not accumulate. The same phenotype is observed in the constitutively stressed *ssa1 ssa2* mutant, which lacks two cytoplasmic members of the hsp70 family of chaperones. Ty3 VLPs preformed under nonstress conditions are degraded more rapidly if cells are shifted from 30°C to 37°C. These results suggest that Ty3 VLPs are destroyed by cellular stress proteins. Elevated expression of the yeast *UBP3* gene, which encodes a protease that removes ubiquitin from proteins, allows mature Ty3 proteins and VLPs to accumulate in the *ssa1 ssa2* mutant, suggesting that, at least under stress conditions, ubiquitination plays a role in regulating Ty3 transposition.**

All organisms respond to elevated temperature and certain other environmental stresses by synthesizing a specific set of proteins called heat shock or stress proteins (1). Many of these stress proteins are members of universally conserved protein families and act as molecular chaperones or components of proteolytic systems. Some stress proteins and their nonstress-induced cognates play fundamental roles in the cell that are not limited to times of stress. These roles include translocating proteins across the mitochondrial and endoplasmic reticulum membranes, protein folding, and protein degradation (1).

There is a long-established relationship between the stress response and viral infection. Stress in the form of heat shock promotes gene expression for some viruses (2) and transposons (3–6). In other instances, heat shock inhibits viral multiplication by a variety of different mechanisms (2).

Many viruses stimulate synthesis of host cell stress proteins during infection. At early times of HIV-1 infection, hsp70 undergoes a relocalization from the cytoplasm to the nucleus (7). Later there is an increase in the overall level of hsp70 within these cells (8). Induction of particular stress proteins or their mRNAs has also been observed during infections by adenoviruses (9), herpesviruses (10–14), paramyxoviruses (15–17), poxviruses (18), papovaviruses (19), togaviruses (20), and rhabdoviruses (20, 21). In most cases, the stress proteins induced are of the hsp60, hsp70, or hsp90 families of chaperone proteins. However, herpesvirus infection also increases transcription of ubiquitin, a stress-inducible component of a proteolytic system (22).

Stress proteins often associate with individual viral proteins, core particles, or virions. Members of the hsp70 protein family are associated with adenovirus fiber protein (23) and picornavirus capsid precursors (24) and coimmunoprecipitate with vaccinia virus proteins (18). Cyclophilin A, a peptidylprolyl *cis-trans* isomerase, is incorporated into HIV-1 virions and this incorporation has been correlated with infectivity of virions (25, 26). Cyclophilins have protein folding activity (27) and are stress-inducible (28). Ubiquitin has been found associated with avian leukosis virus core particles (29), as well as virions of baculovirus (30) and African swine fever virus (31). In addition, ubiquitin has also been found conjugated to structural proteins of plant viruses (32, 33). At least one virus, Sindbis virus, regulates the concentration of its RNA polymerase in part by ubiquitin-mediated proteolysis (34).

Although in eukaryotes roles for stress proteins in viral life cycles have been hypothesized, only the association of cyclophilin with HIV-1 virions (25, 26) and the conjugation of ubiquitin to Sindbis virus RNA polymerase (34) have been shown to be of functional significance. In prokaryotes, however, specific roles for stress proteins in bacteriophage growth have been demonstrated (35). DnaK (an hsp70 protein) and the associated GrpE protein are required for λ DNA replication while GroES and GroEL (hsp10 and hsp60 proteins, respectively) are required for coat assembly of λ, T4, and T5.

We examined the effects of inducing the cellular stress response on transposition of the Ty3 retrovirus-like element of *Saccharomyces cerevisiae*. Ty3 is similar to retroviruses in its genomic structure and life cycle (36–38). Ty3 is transcribed from a provirus-like genomic copy of itself and virus-like particles (VLPs) are assembled that contain Ty3 proteins and genomic RNA. A DNA copy is reverse transcribed from this RNA and integrated back into the host cell genome. Ty3 contains two open reading frames (ORFs), *GAG3* and *POL3*, flanked by long terminal repeats (36, 37) (Fig. 1). The 38-kDa primary translation product of the *GAG3* ORF, $Pr38^{GAG3}$, is processed by the 16 kDa Ty3-encoded protease (PR) (39) into capsid (CA), the 26-kDa major structural protein of the Ty3 VLP, and the 9-kDa nucleocapsid. The 173-kDa primary translation product of *GAG3-POL3*, $Pr173^{GAG3\text{-}POL3}$, is processed into CA, an 11-kDa form of nucleocapsid, PR, the 55-kDa reverse transcriptase (RT), and the 61-kDa and 58-kDa forms of integrase (IN).

Investigating the effects of stress on the yeast Ty3 element will lead to the identification of cellular genes that mediate these effects and an understanding of the roles that stress proteins play in the Ty3 life cycle. Mammalian homologs of these genes may play roles in viral infections of animal cells and

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Abbreviations: VLP, virus-like particles; WCE, whole cell extracts; CA, capsid; PR, protease; RT, reverse transcriptase; IN, integrase.

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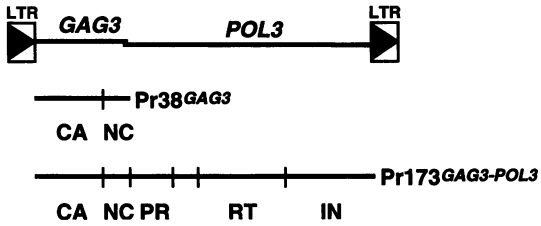

FIG. 1. Ty3 structure and protein products. The genome of Ty3 is depicted above, with the *GAG3* and *POL3* ORFs indicated as well as the long terminal repeats (LTRs). The $Pr38^{GAG3}$ and $Pr173^{GAG3\text{-}POL3}$ primary translation products and the proteins processed from them are depicted below. NC, nucleocapsid.

knowledge gained from the Ty3 system may uncover new approaches for antiviral therapy.

MATERIALS AND METHODS

Strains, Plasmids, Media, and General Procedures. Yeast strains yTM443 (*MATa ura3–52 trp1-H3 his3-Δ200 ade2–101 lys2–1 leu1–12 can1–100 bar1::hisG* Ty3 null) (40) and yTM493 (*MATa ura3–52 trp1-H3 his3-Δ200 ade2–101 lys2–1 leu1–12 can1–100 bar1::hisG ssa1::HIS3 ssa2::URA3* Ty3 null) were used. Strain yTM493 was constructed from yTM443 by transformation with plasmids to disrupt the *SSA1* and *SSA2* genes (see below). *Escherichia coli* strain HB101 was used for plasmid DNA manipulations.

Plasmids pEC450 and pEC452 (41) were used to disrupt the *SSA1* and *SSA2* genes, respectively, by linearization of the plasmid DNA followed by transformation, resulting in gene replacement. Plasmid pTM1155 contains the yeast *UBP3* gene, a 2 μ origin of replication (high copy), and *URA3* and *LYS2* as selectable markers. Plasmid pTM1155 was constructed from pUBP3 (a gift from E. Craig, University of Wisconsin) by inserting the yeast *LYS2* gene on a *Sal*I fragment, removed from pDP6 (42), into the *Sal*I site in pUBP3. Plasmid pTM45 (40) carries in low copy number (ARS/CEN) a galactose-regulated Ty3 element and the yeast *TRP1* gene as a selectable marker. Plasmid pDLC374 (40, 43) carries in high copy number (2μ) a modified tRNA gene as a Ty3 transposition target and the yeast *HIS3* gene as a selectable marker.

SCR is synthetic complete medium (40, 44) containing 2% raffinose (a carbon source that is nonrepressing and noninducing for galactose-regulated promoters), 2% glycerol, and 3% lactic acid. Appropriate nutrients are left out of the SCR medium (e.g., -his-trp = lacking histidine and tryptophan) to allow for selection of plasmid markers.

Southern and Northern blot hybridizations were performed using standard methods (45). Immunoblot analysis of proteins was performed as described previously (40), using the ECL system for detection (Amersham). Antibodies to Ty3 CA and IN proteins and to a peptide corresponding to a region of the Ty3 RT protein have been described (40).

Ty3 Transposition Assay. Nucleic acid samples from yeast cells containing pTM45 and pDLC374 were analyzed for Ty3 transposition using a physical assay previously described (40). Ty3 integrates exclusively at transcriptional start sites of genes that are transcribed by RNA polymerase III (43, 46), namely the genes encoding tRNAs, 5S RNA, and U6 RNA. Ty3 insertion events upstream of the tRNA gene on pDLC374 were amplified by PCR using 25 ng DNA per reaction from total nucleic acid preparations. A PCR product of 474 bp generated with Ty3-specific primers indicates Ty3 insertions at the major transcriptional start site of the tRNA gene on pDLC374. Parallel PCRs with *HIS3*-specific primers amplify a 240-bp segment of the *HIS3* gene on pDLC374 and serve as controls for the presence of the plasmid in similar amounts in all the reactions. All reactions were carried out such that PCR product levels were proportional to the amount of input DNA.

Preparation of Whole Cell Extracts (WCEs), VLPs, and Nucleic Acids. WCEs were prepared by agitating cells in the presence of glass beads as described (40). Ty3 VLPs, containing Ty3 proteins and nucleic acids, were purified by spinning cell extracts through a sucrose step gradient as described (38) with the following modifications. After harvesting cells and washing once in VLP buffer A, cells were resuspended in VLP buffer B and immediately lysed by agitating in the presence of glass beads. Total nucleic acid was prepared by agitating cells in the presence of glass beads and phenol followed by ethanol precipitation of the aqueous phase and resuspension of the nucleic acids. Nucleic acid samples were treated with RNase A before Southern blot hybridization analysis and with DNAse I before Northern blot hybridization analysis. The DNA concentration in nucleic acid samples was determined fluorometrically in the presence of the DNA-specific dye Hoechst 33258 (Calbiochem) using a Mini TKO 100 DNA Fluorometer (Hoefer). The RNA concentration in nucleic acid samples was determined spectrophotometrically by absorbance at 260 nm.

RESULTS

Ty3 Does not Transpose Under Conditions that Induce the Cellular Stress Response. The effect of inducing the stress response on Ty3 transposition was determined by raising the culture temperature of cells expressing Ty3 from 30°C to 37°C (47) or adding 1.55 M ethanol to a culture growing at 30°C (48). Cells of strain yTM443 containing pTM45, which carries a galactose-regulated Ty3 element, and pDLC374, which carries a modified tRNA gene as a Ty3 transposition target, were grown to early logarithmic phase (A_{600} = 0.2) in SCR-his-trp medium. The culture was divided into four portions and galactose (2%) was added to three to induce expression of Ty3. Two of the galactose-containing portions were returned to growth under conditions that induce the cellular stress response: one was incubated at 37°C and the other was incubated at 30°C after adding ethanol to a final concentration of 1.55 M. The remaining two portions were returned to growth at 30°C (nonstress) and served as positive (plus galactose) and negative (no galactose) controls. After 6 h (two generations), cells were harvested and nucleic acid was prepared from each sample. Transposition in the four cultures was measured by a physical assay that amplifies Ty3 insertion events upstream of the tRNA gene on pDLC374 by PCR. As a control for the amount of target plasmid in the reaction, parallel PCRs were performed that amplify *HIS3* sequences on pDLC374. PCR products were separated by PAGE on 6% gels and visualized by fluorescence after staining with ethidium bromide. As shown in Fig. 2*A*, Ty3 transposed in the galactose-treated culture grown at 30°C but not in the cultures where the stress response had been induced.

To determine if Ty3 was transcribed under the various conditions described above, Northern blot analysis was performed on nucleic acid samples from each of the four cultures. RNA was hybridized sequentially with probes to the internal region of Ty3 and the yeast actin gene (*ACT1*), which served as a control for message levels. As shown in Fig. 2*B*, steady state levels of Ty3 RNA were similar under all conditions where Ty3 transcription was induced by addition of galactose.

The Ty3 Life Cycle Is Blocked At or Before VLP Formation During the Cellular Stress Response. The reason that Ty3 is unable to transpose during the stress response was investigated by determining what Ty3 components accumulate under these conditions. Proteins from WCEs prepared from samples of the cultures described above were analyzed by immunoblot, probing with anti-CA antibody. As shown in Fig. 3*A*, only $Pr38^{GAG3}$ is detected in cells undergoing the stress response. The 26-kDa mature CA protein does not accumulate in these cells, suggesting that the Ty3 life cycle is blocked at or before VLP

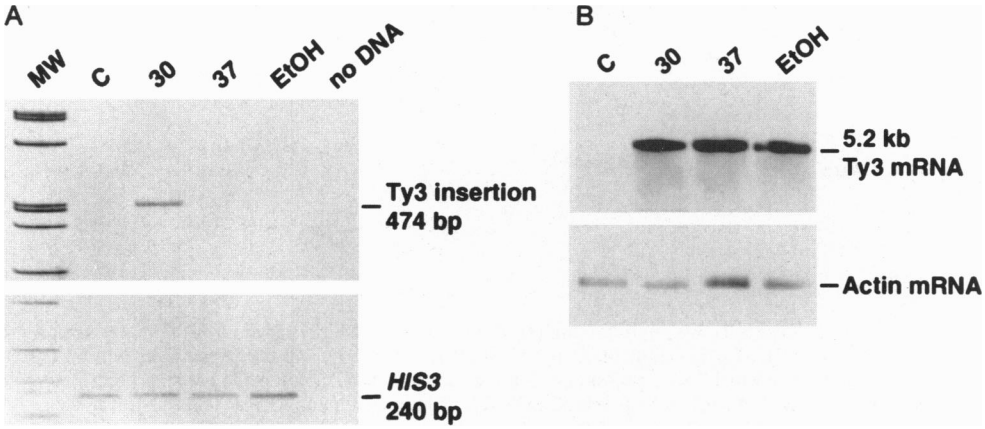

FIG. 2. Effect of stress on Ty3 replication. (*A*) Ty3 transposition measured by PCR. A product of 474 bp indicates Ty3 insertions in the target plasmid. Ty3 transposition was measured at 30°C (30), 37°C (37), or at 30°C in the presence of 1.55 M ethanol (EtOH); C, negative control, where Ty3 expression was not induced in cells growing at 30°C. Below are parallel PCRs that amplify a 240-bp segment of the *HIS3* gene on the target plasmid to indicate equivalent amounts of plasmid were present in all reactions. MW is λ DNA digested with *Pst*I. The no DNA lanes contain PCRs carried out with no template DNA. (*B*) Northern blot hybridization analysis showing steady state levels of Ty3 mRNA under the culture conditions described in *A*. RNA (20 μg per lane) from total nucleic acid samples described in *A* was used. As a control for the Ty3 message level, the membrane was stripped and reprobed for actin mRNA.

formation. $Pr173^{GAG3\text{-}POL3}$ was not detected in stressed cells. However, $Pr173^{GAG3\text{-}POL3}$ is not even detectable under transposition-permissive conditions, suggesting that it is a very unstable protein. The nucleic acid samples described above were analyzed by Southern blot hybridization, probing with an internal portion of Ty3 DNA. The 5.4-kbp species of Ty3 DNA, the product of reverse transcription of Ty3 genomic RNA, was not detected in cells undergoing the stress response (Fig. 3*B*), consistent with a block at an early stage of the Ty3 life cycle.

A Ty3 phenotype was also assessed in cells in which the stress response was induced not environmentally, but genetically. Ty3 was expressed in a *ssa1 ssa2* mutant strain (yTM493) derived from yTM443. *SSA1* and *SSA2* encode two cytoplasmic members of the hsp70 family of stress proteins (49). Under nonstress conditions, the *ssa1 ssa2* mutant is constitutively thermotolerant and expresses many stress proteins (41, 50). Cells of strain yTM493 transformed with pTM45 were grown at 30°C in SCR-trp to early logarithmic phase. The culture was split into two portions and galactose was added to one portion. Incubation was resumed for 6h, at which point WCEs were prepared and analyzed by immunoblot. As shown in Fig. 3*A*, mature CA was not formed in the *ssa1 ssa2* mutant expressing Ty3, although $Pr38^{GAG3}$ could be detected.

Cultures of yTM443 transformed with pTM45 and pDLC374 grown under Ty3-inducing conditions for 6 h at 30°C, 37°C, and at 30°C in the presence of 1.55 M ethanol and cultures of yTM493 transformed with pTM45 grown under Ty3-inducing conditions for 6 h at 30°C were analyzed for VLPs. As shown in Fig. 4, mature CA, IN, and RT were detected in VLP preparations from control cells but not cells incubated at 37°C; likewise, mature CA was not detected in VLP preparations from cells grown in the presence of 1.55 M ethanol or from the *ssa1 ssa2* mutant. Thus, mature Ty3 VLPs did not accumulate in stressed cells or the *ssa1 ssa2* mutant. However, $Pr38^{GAG3}$ was recovered in particle preparations from stressed cells.

Preformed VLPs Turn Over More Rapidly at 37°C Versus 30°C. The preceding experiments established that the stress response induced by physical or genetic means interferes with the accumulation of Ty3 proteins and VLPs. To determine whether there is an effect of the stress response on the integrity of preformed particles, the stability of VLPs in control cells and cells undergoing the stress response was determined. Strain yTM443, transformed with pTM45 and pDLC374, was grown to early log phase in SCR-his-trp at 30°C. The culture was split and one portion was returned to 30°C. Galactose (2%) was added to the second portion and it was returned to 30°C. After 6 h, glucose (2%) was added to the galactose-containing portion for 30 min to repress Ty3 transcription. These cells were washed twice with SCR-his-trp plus 2% glucose, resuspended in this medium, and split into two further portions. One portion was incubated at 30°C, the other portion at 37°C. At intervals, samples were removed from each culture and analyzed by immunoblot, probing with anti-CA antibody, and by Southern blot hybridization, probing with an internal por-

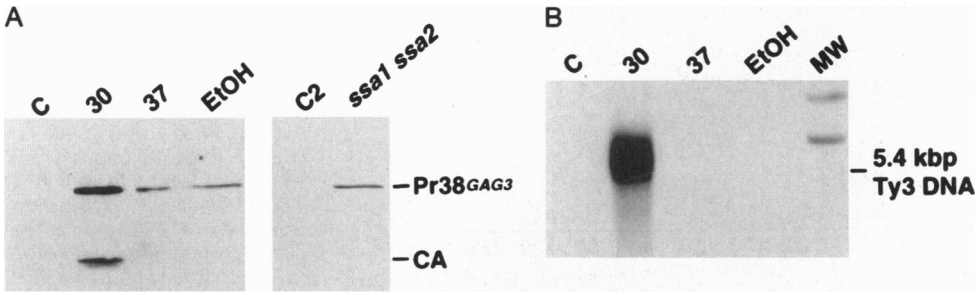

FIG. 3. Ty3 components present in stressed cells. (*A*) Immunoblot analysis of proteins (3 μg per sample) in WCEs from the cultures described in Fig. 2. Blots were probed with anti-CA antibody, which detects CA, $Pr38^{GAG3}$, and a 31-kDa protein thought to be an intermediate cleavage product of $Pr38^{GAG3}$ (not detected on this immunoblot). Immunoblot analysis, probing with anti-CA antibody, was also done using protein samples from the *ssa1 ssa2* mutant transformed with pTM45 left untreated at 30°C (C2) or incubated for 6 h in the presence of 2% galactose at 30°C (*ssa1 ssa2*). (*B*) Southern blot hybridization analysis of DNA (100 ng per lane) from cultures grown as described in Fig. 2. The probe detects the product of Ty3 reverse transcription, a 5.4-kbp DNA. MW is λ DNA digested with *Hin*dIII.

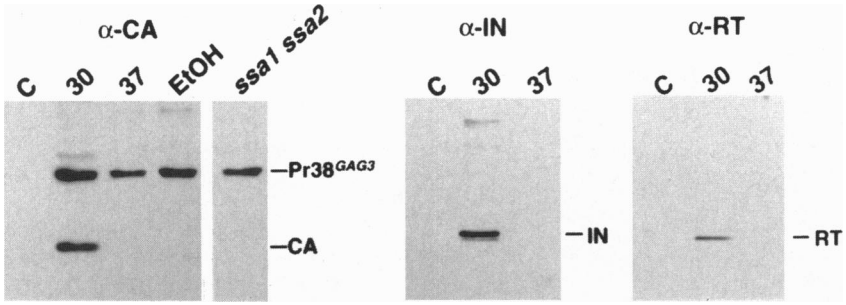

FIG. 4. Analysis of Ty3 VLPs in stressed cells. Immunoblot analysis of proteins in VLP preparations (500 ng per sample) from cultures of wild type (yTM443) expressing Ty3 at 30°C (30), 37°C (37), and 30°C in 1.55 M ethanol (EtOH), and of the *ssa1 ssa2* mutant (yTM493) expressing Ty3 (*ssa1 ssa2*). C, negative control: wild type in which Ty3 expression was not induced. Anti-CA detects the proteins described in Fig. 3, anti-IN detects the 61-kDa and 58-kDa IN proteins, while anti-RT detects the 55-kDa RT protein and a 115-kDa RT–IN fusion not visible in this blot.

tion of Ty3 DNA. As shown in Fig. 5, both Ty3 protein and DNA turn over more rapidly when cells are incubated at 37°C than when they are incubated at 30°C. Shifting cells containing preformed VLPs into medium containing 1.55 M ethanol similarly resulted in more rapid turnover of VLPs compared with shifting cells into medium without ethanol, although the difference was less dramatic than that observed following the temperature shift (unpublished data).

Increased Copy Number of *UBP3* Allows Mature VLPs to Accumulate in the *ssa1 ssa2* Mutant. In yeast, stress increases expression of some components of the ubiquitin-mediated proteolytic system (51, 52). Because overexpression of the yeast *UBP3* gene, which encodes a ubiquitin processing protease (53), has been shown to suppress the temperature sensitivity of the *ssa1 ssa2* mutant (50), we tested the effect of overexpressing *UBP3* on Ty3 protein and VLP formation. Transformants of yTM493 carrying pTM45 or pTM45 and pTM1155, a high-copy plasmid carrying the yeast *UBP3* gene, were grown to early logarithmic phase at 30°C in SCR-trp and SCR-trp-lys, respectively. Galactose (2%) was added to both cultures and incubation was continued for 12 h, at which point WCEs were prepared for immunoblot analysis. Total nucleic acid was extracted from parallel samples of the above cultures and analyzed by Southern blot hybridization for the presence of replicated Ty3 DNA. As shown in Fig. 6*A*, the 26-kDa mature CA protein and replicated Ty3 DNA accumulated in the *ssa1 ssa2* mutant containing *UBP3* on a high-copy plasmid.

Cultures of yTM493 carrying pTM45 or pTM45 and pTM1155 grown as above were also analyzed for VLPs. As shown in Fig. 6*B*, mature Ty3 VLPs form when *UBP3* is present in high copy as evidenced by the accumulation of mature CA in the particulate fraction of the cell.

DISCUSSION

Ty3 does not transpose when cells are stressed. *In vitro* studies of Ty3 RT (38) showed that its activity was reduced to near background levels at 37°C and the lack of Ty3 DNA in cells growing at 37°C is consistent with this observation. Ethanol treatment of cultures at 30°C had the same effect on Ty3 as raising the temperature to 37°C, raising the possibility that Ty3 RT is damaged by these stresses. However, analysis of Ty3 proteins from stressed cells indicated that the block of Ty3 transposition is earlier in the life cycle than reverse transcription, occurring at or before VLP formation. By analogy to retroviruses, processing of the Ty3 polyproteins $Pr38^{GAG3}$ and $Pr173^{GAG3\text{-}POL3}$ is believed to occur in the context of a Ty3 particle, where Ty3 protease can reach concentrations sufficient to form enzymatically active dimers (54). The lack of mature Ty3 proteins in stressed cells is consistent with a defect in VLP formation in these cells.

Heat and ethanol treatments may damage cellular or Ty3 proteins (other than RT), leading to the effects on Ty3 observed during these stresses. Alternatively, cellular proteins associated with the stress response could be mediating these effects, independent of changes in the Ty3 particle, a possibility supported by experiments with the *ssa1 ssa2* mutant. Specifically, induction of the stress response genetically, in the

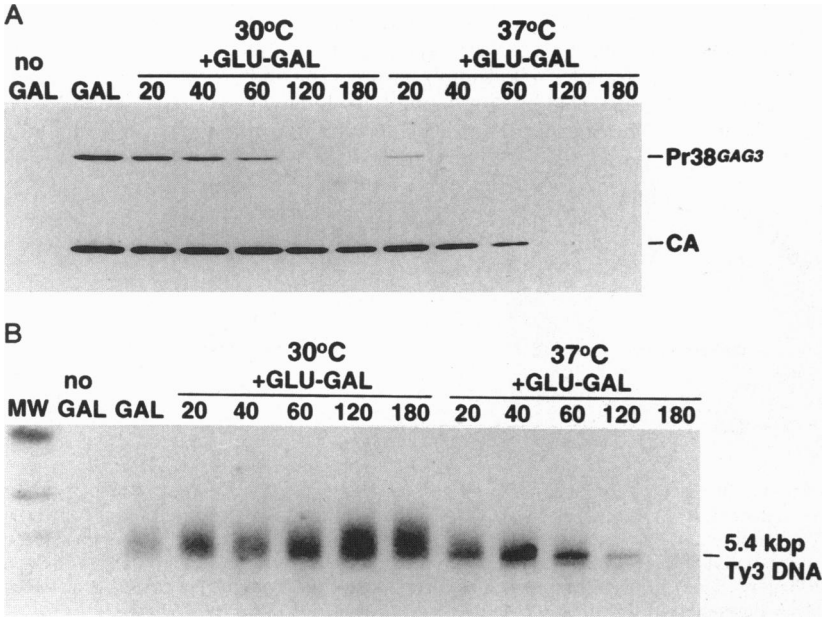

FIG. 5. Fate of mature Ty3 VLP components. Protein and nucleic acid samples were prepared from cultures of yTM443 transformed with pTM45 and pDLC374 grown under the following conditions: untreated and grown at 30°C (no GAL); incubated with 2% galactose for 6 h at 30°C (GAL); incubated with 2% galactose for 6 h at 30°C, harvested, then washed and resuspended in glucose medium (with no galactose) for 20–180 min at 30°C (20–180 min under 30°C heading); incubated with 2% galactose for 6 h at 30°C, harvested, then washed and resuspended in glucose medium (with no galactose) for 20–180 min at 37°C (20–180 min under 37°C heading). (*A*) Immunoblot analysis of protein samples (3 μg per lane) using anti-CA antibody. (B) Southern blot hybridization analysis (100 ng of DNA per lane) probing for the product of Ty3 reverse transcription. MW is λ DNA digested with *Hin*dIII.

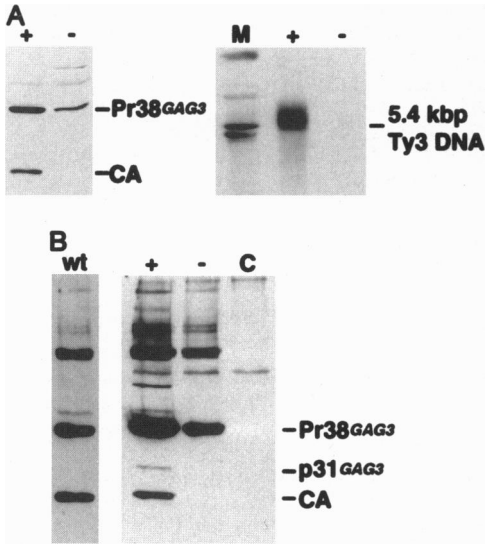

FIG. 6. Effect of overexpression of *UBP3* on Ty*3* in *ssa1 ssa2* mutant cells. (*A*) On the left is an immunoblot of WCEs (5 μg of protein per sample) from cultures of the *ssa1 ssa2* mutant expressing Ty*3* at 30°C for 12 h and either containing (+) or not containing (-) the *UBP3* plasmid. On the right is Southern blot hybridization analysis of DNA (1 μg per sample) from the same cultures, probing for the product of Ty*3* reverse transcription. MW is λ DNA digested with *Hin*dIII. (*B*) Immunoblot analysis of proteins in VLP preparations (500 ng of protein per sample) from cultures of the *ssa1 ssa2* mutant expressing Ty*3* at 30°C for 12 h and either containing (+) or not containing (-) the *UBP3* plasmid. C, negative control, in which Ty*3* expression was not induced. The lane to the left (wt) is a parallel immunoblot of a VLP preparation from wild type expressing Ty*3* at 30°C for 12 h.

absence of physical damage to Ty*3* proteins, is sufficient for generating the Ty*3* stress phenotype. If all the methods of inducing the stress response act in a common way to block Ty*3* transposition, then induction or repression of cellular factors during stress blocks the accumulation of mature Ty*3* VLPs. This phenotype could be caused by the inability of precursors to associate, lability of associating precursors, lability of newly formed mature particles, or inactivity of Ty*3* PR. Because $Pr38^{GAG3}$ was recovered in particle preparations from stressed cells, this suggests that precursors associate in these cells and may participate in early stages of VLP formation. However, because the level of precursor protein recovered in WCEs of stressed cells is reduced compared with nonstressed cells it is still possible that precursor interactions are decreased during stress. A defect in PR during stress is not likely if the defect in VLP accumulation during stress is related to the more rapid turnover of preformed VLPs at 37°C compared with 30°C because preformed VLPs presumably no longer require the activity of PR. However, if gene products encoded by *POL3* (such as PR) are underrepresented in the precursor interactions observed in stressed cells, then a processing defect could result. A defect in ribosomal frameshifting from the *GAG3* ORF to the *POL3* ORF, required to generate $Pr173^{GAG3\text{-}POL3}$, could cause such a decrease in the level of *POL3*-encoded products.

We further explored the possibility that Ty*3* VLPs and precursors are labile and undergo proteolysis during the stress response. In yeast, stress increases expression of components of the ubiquitin-mediated proteolytic system: *UBC4* and *UBC5* (51), genes for ubiquitin conjugating enzymes, and *UBI4* (52), a gene encoding a polyprotein of five tandemly arranged ubiquitin moieties. Craig *et al.* (50) have already shown that overexpression of the yeast *UBP3* gene suppresses the temperature sensitivity of the *ssa1 ssa2* mutant. *UBP3* encodes a ubiquitin processing protease that removes ubiquitin from proteins to which it has been conjugated (53), an activity antagonistic to ubiquitin-mediated proteolysis. When carried in high copy number, the *UBP3* gene suppressed the defect in the formation of mature Ty*3* proteins and VLPs in the *ssa1 ssa2* mutant. Furthermore, the presence of Ty*3* DNA in cells carrying the *UBP3* plasmid indicates that the suppression extends to events in the Ty*3* life cycle subsequent to VLP formation. Therefore, at least for the *ssa1 ssa2* mutant, the inability to accumulate mature VLPs is likely the only block in the Ty*3* life cycle. Reversion of the Ty*3* stress phenotype by elevated expression of an activity antagonistic to ubiquitination suggests a role for ubiquitin conjugation in blocking Ty*3* transposition during stress. Ubiquitin could be conjugated to Ty*3* or cellular proteins and mediate their destruction by the proteosome. With respect to Ty*3* proteins, it is clear how such a process could block transposition; for cellular proteins, proteolysis of a VLP assembly/stability factor, for example, could have the observed effect. We are currently analyzing Ty*3* VLPs for the presence of ubiquitin and ubiquitin conjugates.

The *UBP3* plasmid did not complement the Ty*3* defect in the formation of mature VLP proteins and, hence, transposition in wild-type cells incubated at 37°C (unpublished data). Formation of mature VLPs is epistatic to reverse transcription and integration. In the *ssa1 ssa2* mutant, although expression of many stress proteins is increased, such is not the case for *UBC4*, *UBC5*, or *UBI4* (50). However, hsp70 levels are reduced in the *ssa1 ssa2* mutant (50) and the balance between chaperone activity and proteolysis could therefore still be shifted toward proteolysis. Because, unlike the *ssa1 ssa2* mutant, proteolytic components are expressed at elevated levels in cells incubated at 37°C, the ubiquitin protease activity generated from the plasmid may not be sufficient to antagonize proteolysis.

These results describe one of the first instances of degradation by the stress response of a native protein. Protein multimers formed during the early stages of Ty*3* VLP morphogenesis, as well as mature VLPs, may be recognized as aggregates by the protein degradation machinery present during the stress response. In wild-type cells at 30°C, this activity is attenuated and mature Ty*3* VLP formation is sufficient for transposition to occur.

As described, ubiquitin has been found physically associated with the proteins or core particles of many viruses (29, 32, 33). Our experiments demonstrate that accumulation of Ty*3* VLPs is sensitive to levels of ubiquitination. The fact that several viruses actually encode ubiquitin, ubiquitin-like proteins, or ubiquitin conjugating enzymes (55–57) suggests that the ubiquitin system may play an additional, positive role in the viral life cycle (e.g., uncoating), although this remains to be determined.

We thank E. Craig for providing plasmids to create the *ssa1 ssa2* strain and for the high copy *UBP3* plasmid. T.M.M. was funded by a postdoctoral fellowship from the American Cancer Society, Public Health Service Training Grant 5 T32 AI07319 from the National Institutes of Health, and the Cancer Research Coordinating Committee of the University of California. S.B.S. was funded by American Cancer Society Grant VM10.

1. Morimoto, R. I., Tissieres, A. & Georgopoulos, C. (1994) in *The Biology of Heat Shock Proteins and Molecular Chaperones*, eds. Morimoto, R. I., Tissieres, A. & Georgopoulos, C. (Cold Spring Harbor Lab. Press, Plainview, NY), pp. 1–30.
2. Nover, L. (1991) in *Heat Shock Response*, ed. Nover, L. (CRC, Boca Raton, FL), pp. 473–482.
3. Zuker, C., Cappello, J., Lodish, H. F., George, P. & Chung, S. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 2660–2664.
4. Strand, D. J. & McDonald, J. F. (1985) *Nucleic Acids Res.* **13**, 4401–4410.
5. Junakovic, N., Di Franco, C., Barsanti, P. & Palumbo, G. (1986) *J. Mol. Evol.* **24**, 89–93.
6. Ratner, V. A., Zabanov, S. A., Kolesnikova, O. V. & Vasilyeva, L. A. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 5650–5654.

7. Furlini, G., Vignoli, M., Re, M. C., Gibellini, D., Ramazzotti, E., Zauli, G. & La Placa, M. (1994) *J. Gen. Virol.* **75,** 193–199.
8. Poccia, F., Placido, R., Mancino, G., Mariani, F., Ercoli, L., di Cesare, S. & Colizzi, V. (1993) in *New Concepts in AIDS Pathogenesis*, eds. Montagnier, L. & Gougeon, M. (Dekker, New York), pp. 195–218.
9. Nevins, J. R. (1982) *Cell* **29,** 913–919.
10. Notarianni, E. L. & Preston, C. M. (1982) *Virology* **123,** 113–122.
11. Macnab, J. C., Orr, A. & La Thangue, N. B. (1985) *EMBO J.* **4,** 3223–3228.
12. LaThangue, N. B. & Latchman, D. S. (1988) *Exp. Cell. Res.* **178,** 169–179.
13. LaThangue, N. B., Shriver, K., Dawson, C. & Chan, W. L. (1984) *EMBO J.* **3,** 267–277.
14. Colberg-Poley, A. M. & Santomenna, L. D. (1988) *Virology* **166,** 217–228.
15. Hightower, L. & Smith, M. (1978) in *Negative Strand Viruses and the Host Cell*, eds. Mahy, B. & Barry, R. (Academic, London), pp. 395–405.
16. Collins, P. L. & Hightower, L. E. (1982) *J. Virol.* **44,** 703–707.
17. Peluso, R. W., Lamb, R. A. & Choppin, P. W. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 6120–6124.
18. Jindal, S. & Young, R. A. (1992) *J. Virol.* **66,** 5357–5362.
19. Khandjian, E. W. & Turler, H. (1983) *Mol. Cell. Biol.* **3,** 1–8.
20. Garry, R. F., Ulug, E. T. & Bose, H. R., Jr. (1983) *Virology* **129,** 319–332.
21. Sagara, J. & Kawai, A. (1992) *Virology* **190,** 845–848.
22. Kemp, L. M. & Latchman, D. S. (1988) *Virology* **166,** 258–261.
23. Macejak, D. G. & Luftig, R. B. (1991) *Virology* **180,** 120–125.
24. Macejak, D. G. & Sarnow, P. (1992) *J. Virol.* **66,** 1520–1527.
25. Franke, E. K., Yuan, H. E. & Luban, J. (1994) *Nature (London)* **372,** 359–362.
26. Thali, M., Bukovsky, A., Kondo, E., Rosenwirth, B., Walsh, C. T., Sodroski, J. & Gottlinger, H. G. (1994) *Nature (London)* **372,** 363–365.
27. Lang, K., Schmid, F. X. & Fischer, G. (1987) *Nature (London)* **329,** 268–270.
28. Parsell, D. & Lindquist, S. (1994) in *The Biology of Heat Shock Proteins and Molecular Chaperones,* eds. Morimoto, R., Tissieres, A. & Georgopoulos, C. (Cold Spring Harbor Lab. Press, Plainview, NY), pp. 457–494.
29. Putterman, D., Pepinsky, R. B. & Vogt, V. M. (1990) *Virology* **176,** 633–637.
30. Guarino, L. A., Smith, G. & Dong, W. (1995) *Cell* **80,** 301–309.
31. Hingamp, P. M., Leyland, M. L., Webb, J., Twigger, S., Mayer, R. J. & Dixon, L. K. (1995) *J. Virol.* **69,** 1785–1793.
32. Dunigan, D. D., Dietzgen, R. G., Schoelz, J. E. & Zaitlin, M. (1988) *Virology* **165,** 310–312.
33. Hazelwood, D. & Zaitlin, M. (1990) *Virology* **177,** 352–356.
34. de Groot, R. J., Rumenapf, T., Kuhn, R. J., Strauss, E. G. & Strauss, J. H. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 8967–8971.
35. Georgopoulos, C., Ang, D., Liberek, K. & Zylicz, M. (1990) in *Stress Proteins in Biology and Medicine,* eds. Morimoto, R. I., Tissieres, A. & Georgopoulos, C. (Cold Spring Harbor Lab. Press, Plainview, NY), pp. 191–221.
36. Hansen, L. J., Chalker, D. L. & Sandmeyer, S. B. (1988) *Mol. Cell. Biol.* **8,** 5245–5256.
37. Hansen, L. J. & Sandmeyer, S. B. (1990) *J. Virol.* **64,** 2599–2607.
38. Hansen, L. J., Chalker, D. L., Orlinsky, K. J. & Sandmeyer, S. B. (1992) *J. Virol.* **66,** 1414–1424.
39. Kirchner, J., Sandmeyer, S. B. & Forrest, D. B. (1992) *J. Virol.* **66,** 6081–6092.
40. Menees, T. M. & Sandmeyer, S. B. (1994) *Mol. Cell. Biol.* **14,** 8229–8240.
41. Werner-Washburne, M., Stone, D. E. & Craig, E. A. (1987) *Mol. Cell. Biol.* **7,** 2568–2577.
42. Fleig, U. N., Pridmore, R. D. & Philippsen, P. (1986) *Gene* **46,** 237–245.
43. Chalker, D. L. & Sandmeyer, S. B. (1992) *Genes Dev.* **6,** 117–128.
44. Sherman, F., Fink, G. R. & Hicks, J. B. (1986) *Methods in Yeast Genetics* (Cold Spring Harbor Lab. Press, Plainview, NY).
45. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K. (1994) *Current Protocols in Molecular Biology* (Curr. Protocols, Brooklyn, NY).
46. Kirchner, J., Connolly, C. M. & Sandmeyer, S. B. (1995) *Science* **267,** 1488–1491.
47. Walton, E. F. & Pringle, J. R. (1980) *Arch. Microbiol.* **124,** 285–287.
48. Plesset, J., Palm, C. & McLaughlin, C. S. (1982) *Biochem. Biophys. Res. Commun.* **108,** 1340–1345.
49. Craig, E. A. & Jacobsen, K. (1984) *Cell* **38,** 841–849.
50. Craig, E. A., Baxter, B. K., Becker, J., Halladay, J. & Ziegelhoffer, T. (1994) in *The Biology of Heat Shock Proteins and Molecular Chaperones,* eds. Morimoto, R. I., Tissieres, A. & Georgopoulos, C. (Cold Spring Harbor Lab. Press, Plainview, NY), pp. 31–52.
51. Seufert, W. & Jentsch, S. (1990) *EMBO J.* **9,** 543–550.
52. Finley, D., Ozkaynak, E. & Varshavsky, A. (1987) *Cell* **48,** 1035–1046.
53. Baker, R. T., Tobias, J. W. & Varshavsky, A. (1992) *J. Biol. Chem.* **267,** 23364–23375.
54. Kirchner, J. & Sandmeyer, S. (1993) *J. Virol.* **67,** 19–28.
55. Meyers, G., Tautz, N., Dubovi, E. J. & Thiel, H. J. (1991) *Virology* **180,** 602–616.
56. Hingamp, P. M., Arnold, J. E., Mayer, R. J. & Dixon, L. K. (1992) *EMBO J.* **11,** 361–366.
57. Russell, R. L. & Rohrmann, G. F. (1993) *J. Gen. Virol.* **74,** 1191–1195.