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A Truncated Form of KlLsm4p and the Absence of Factors Involved in mRNA Decapping Trigger Apoptosis in Yeast

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The *LSM4* gene of *Saccharomyces cerevisiae* codes for an essential protein involved in pre-mRNA splicing and also in mRNA decapping, a crucial step for mRNA degradation. We previously demonstrated that the first 72 amino acids of the *Kluyveromyces lactis* Lsm4p (KlLsm4p), which contain the Sm-like domains, can restore cell viability in both *K. lactis* and *S. cerevisiae* cells not expressing the endogenous protein. However, the absence of the carboxy-terminal region resulted in a remarkable loss of viability in stationary phase cells (Mazzoni and Falcone, 2001). Herein, we demonstrate that *S. cerevisiae* cells expressing the truncated *LSM4* protein of *K. lactis* showed the phenotypic markers of yeast apoptosis such as chromatin condensation, DNA fragmentation, and accumulation of reactive oxygen species. The study of deletion mutants revealed that apoptotic markers were clearly evident also in strains lacking genes involved in mRNA decapping, such as *LSM1*, *DCP1*, and *DCP2*, whereas a slight effect was observed in strains lacking the genes *DHH1* and *PAT1*. This is the first time that a connection between mRNA stability and apoptosis is reported in yeast, pointing to mRNA decapping as the crucial step responsible of the observed apoptotic phenotypes.

INTRODUCTION

Apoptosis is a kind of programmed cell suicide crucial for health, homeostasis, and embryonic development. Its important role in different diseases such as cancer, neurodegenerative disorders, or stroke, and its very complex regulatory network were discovered in model organisms such as *Drosophila melanogaster* or *Caenorhabditis elegans*. Recent studies support the notion that apoptosis also occurs in *Saccharomyces cerevisiae* (Frohlich and Madeo, 2000; Madeo *et al.*, 2002a). Apoptosis in yeast has been demonstrated in very different cases, e.g., in *cdc48* mutants (Madeo *et al.*, 1997); in cells expressing *Bax*, the mammalian apoptosis inducer gene (Ligr *et al.*, 1998); during perturbation of the vesicular trafficking (Levine *et al.*, 2001) and salt stress (Huh *et al.*, 2002); and after cell treatment with osmotin, an antifungal protein from tobacco implicated in host-plant defense (Narasimhan *et al.*, 2001). Very recently, evidence for the existence of a caspase-related protease regulating apoptosis has been reported in yeast cells (Madeo *et al.*, 2002b). Most of these scenarios were related to oxygen stress, suggesting that reactive oxygen species (ROS) are key regulators of yeast apoptosis (Madeo *et al.*, 1999).

Interestingly, it has also been reported that altered pre-mRNA splicing or mRNA stability are involved in mammalian apoptotic-linked diseases (Guhaniyogi and Brewer, 2001; Nissim-Rafinia and Kerem, 2002).

In previous work (Mazzoni and Falcone, 2001), we demonstrated that the *Kluyveromyces lactis LSM4* gene (*KlLSM4*), and a truncated form (*Kllsm4Δ1*) still containing the Sm-like domains, could restore viability in a *S. cerevisiae* strain not expressing the endogenous Lsm4p, a subunit of the Lsm complex that is involved in mRNA decapping and splicing (Cooper *et al.*, 1995; Tharun *et al.*, 2000). We also reported that cells expressing KlLsm4Δ1p showed an increased loss of viability when reaching the stationary phase and, therefore, we wondered whether the lack of the C-terminal region of Lsm4p could lead to apoptotic death in yeast.

Because most of the reports concerning yeast apoptosis have been done with *S. cerevisiae*, we looked for the cytological markers of apoptosis in an *S. cerevisiae* strain deprived of

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This article is dedicated to Franco Tatò.

Table 1. Yeast strains used in this study

Strain	Genotype	Source
MCY4	*Mat α, ade1-101, his3-Δ1, trp1-289, ura3, LEU-GAL1-SDB23*	Cooper *et al.*, 1995
MCY4/KILSM4	*Mat α, ade1-101, his3-Δ1, trp1-289, ura3, LEU-GAL1-SDB23 pRS416/KILSM4*	Mazzoni and Falcone, 2001
MCY4/Kilsm4Δ1	*Mat α, ade1-101, his3-Δ1, trp1-289, ura3, LEU-GAL1-SDB23, pRS416/Kilsm4Δ1*	Mazzoni and Falcone, 2001
AEMY24	*Mat α ura3-1, leu2-3,-112, ade2-1, can1-100, his3-11,-15, trp1Δ1, lam1Δ::TRP1*	Mayes *et al.*, 1999
YRP840	*Mat* **a**, *ura3-52, trp1, leu2-3,112, his4-539, cup::LEU2(PM)*	Hatfield *et al.*, 1996
YRP1069	*Mat α, ura3-52, trp1, leu2-3,112, cup1::LEU2(PM), lys2-201, dcp1Δ::URA3*	Roy Parker
YRP1346	*Mat* **a**, *ura3-52, trp1, leu2-3,112, his4-539, cup1::LEU2(PM), lys2-201, dcp2::TRP1*	Dunckley and Parker, 1999
YRP1372	*Mat* **a**, *ura3-52, trp1, leu2-3,112, his4-539, cup1::LEU2(PM), pat1Δ::LEU2*	Tharun *et al.*, 2000
YRP1580	*Mat* **a**, *ura3-52, trp1, leu2-3,112, his4-539, cup1::LEU2(PM), lys2-201, dhh1Δ::URA3*	Coller *et al.*, 2001

the endogenous *LSM4* gene and expressing the truncated form of the *K. lactis* gene.

Our results demonstrated that all the hallmarks of apoptosis are present in this strain and indicated that this phenomenon is due an increase in mRNA stability as also confirmed by the analysis of *S. cerevisiae* strains lacking specific components of the mRNA turnover machinery (i.e., *LSM1*, *DCP1*, *DCP2*, *DHH1*, and *PAT1*).

MATERIALS AND METHODS

Strains and Culture Conditions

The yeast strains used in this study are listed in Table 1.

Cells were grown in YP (1% yeast extract, 2% peptone) supplemented with 2% glucose (YPD), 2% galactose (YPGal), or glycerol (YPGly) at 28°C (unless indicated). Solid media were supplemented with 2% bactoagar (Difco, Detroit, MI).

Induction of [*rho*°] was obtained by growing the MCY4/*KlLSM4* and MCY4/*Kllsm4′441* strains for 24 h in the presence of 25 μg/ml ethidium bromide. The [*rho*°] mutants were selected by their inability to grow on respiratory medium (YPGly) followed by observation of 4,6-diamidino-2-phenylindole (DAPI) staining.

Fluorescence Microscopy

For DAPI staining, exponentially growing cells (OD_{600} = 0.5) were harvested, resuspended in 70% (vol/vol) ethanol, stained with DAPI at the concentration of 1 μg/ml, and observed by fluorescence microscopy.

The presence of free intracellular radicals or strongly oxidizing molecules (ROS) were detected with dihydrorhodamine (DHR) (D1054; Sigma-Aldrich, St. Louis, MO) as described previously (Madeo *et al.*, 1999).

Free 3′-OH was detected by terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) (Madeo *et al.*, 1997) by using the Roche Diagnostics (Mannheim, Germany) in situ cell detection kit peroxidase and observed with an Axioscope microscope (Carl Zeiss, Jena, Germany).

Electron Microscopy

Exponentially growing cells (OD_{600} = 4) were fixed with 2% glutaraldehyde in distilled water for 1 h at room temperature and washed with water. To reveal cellular membranes without removing the cell wall, cells were postfixed with freshly prepared 4% $KMnO_4$ in H_2O for 2 h at 4°C (Kaiser and Schekman, 1990). After washes, cells were incubated with 2% uranyl acetate for 2 h at room temperature, washed, and dehydrated in increasing (30–100%) concentrations of ethanol.

The samples were infiltrated overnight at 4°C in a 1:1 mixture of ethanol with Epon 812 embedding medium. The mixture was replaced with pure Epon 812, and the samples were allowed to polymerize at 60°C for 24 h. Ultrathin sections were stained with lead citrate before examination at electron microscope.

RNA Isolation and Analysis

Total RNA was prepared as described previously (Schmitt *et al.*, 1990) and, after spectrophotometric determination of the amount present in each sample, 10 μg of RNA was loaded onto 1.2% agarose-3-(*N*-morpholino)propanesulfonic acid gels containing formaldehyde and ethidium bromide. Northern analysis was performed by standard procedures (Sambrook *et al.*, 1989). Hybridization was carried out at 37°C by using 5′-end–labeled oligonucleotide 5′-GTGGTACGCCTCTTGGAGCGGGTGGAATACCGCTC-3′, complementary to *SSA4* gene (Saavedra *et al.*, 1997) in the presence of 20× SSPE, 50× Denhardt's, and 10% SDS.

To test splicing efficency, 15 μg of total RNA was run onto 6% acrylamide/urea gel and elettroblotted on Hybond-N^+ for 1 h at 60 V in Tris buffer 0.5×, UV cross-linked, and then hybridized by random priming labeled U3 probe, obtained by polymerase chain reaction amplification reaction from two oligonucleotides (5′-CCAACTTGGTTGATGAGTCC-3′; 5′-GGATGGGTCAAGATCATCGC-3′) complementary to the exon2 region of *SNR17* gene (Hughes *et al.*, 1987).

The hybridization was performed at 42°C in 50% formammide, 5× SSC, 10× Denhardt's, 0.3% SDS and 150 μg of single-stranded DNA. After hybridization the membrane was washed for 30 min at 37°C in 2× SSC and 0.2% SDS and then for 2 h at 48°C in 0.1× SSC and 0.4% SDS. As molecular marker, we used pBR322 digested with *Msp*I.

RESULTS

Cells Expressing the Truncated Form Kllsm4Δ1p *Showed Cytological Markers of Apoptosis*

To investigate whether the loss of viability observed in stationary yeast cells expressing Kllsm4Δ1p was related to apoptotic events, we analyzed the cellular and the nuclear morphology. As shown in Figure 1, cells expressing the entire *KlLSM4* gene showed a normal morphology (Figure 1b) and a single round-shaped nucleus was detected in each cell by DAPI staining (Figure 1a), whereas cells expressing the truncated form of the gene revealed the presence of abnormally elongated and misformed cells (Figure 1, d and f). Moreover, ~20% of these cells showed an evident nuclei fragmentation and chromatin margination, leading to the formation of a ring at the inner side of the nuclear envelope (Figure 1, c and e), as also was observed in apoptotic cells of

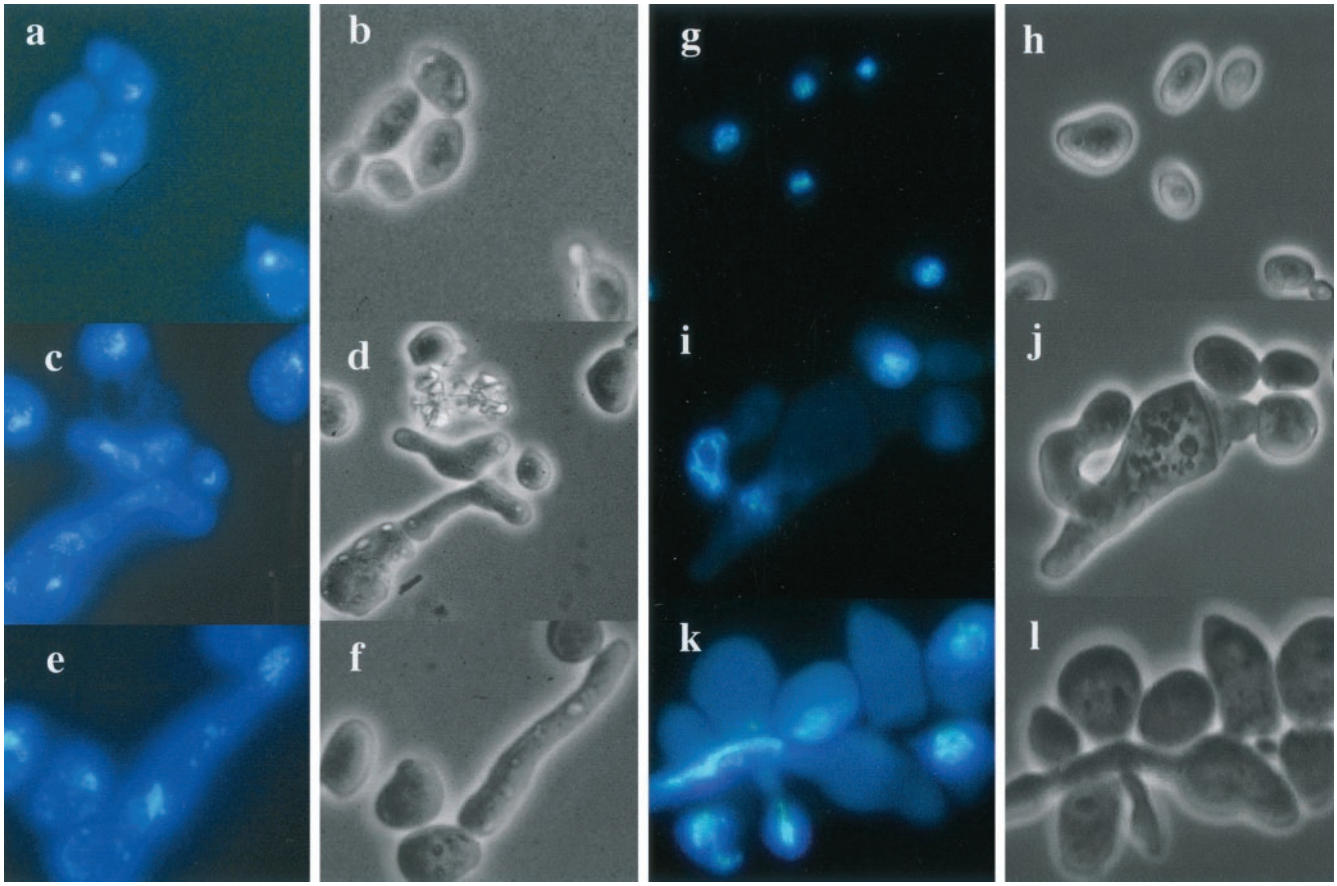

Figure 1. Chromatin fragmentation. DAPI staining and phase contrast of *S. cerevisiae* strain MCY4 containing plasmids pRS416/*KlLSM4* (a and b), pRS416/*Kllsm4*Δ*1* (c–f), and the corresponding [*rho*°] derivatives (*KlLSM4*, g and h; *Kllsm4*Δ*1*, i–l) grown on YPD.

other organisms (Lazebnik *et al.*, 1993), and the number of cells showing these defects increased at higher optical density of the culture.

To be sure that the bright spots revealed with DAPI staining were actually derived from chromatin fragmentation and not due to mitochondrial DNA, we obtained by ethidium bromide treatment [*rho*°] strains from both the *KlLSM4*- and *Kllsm4*Δ*1*-expressing cells (see MATERIALS AND METHODS). As shown in Figure 1, also in the [*rho*°] strains, the expression of the entire gene *KlLSM4* led to a normal morphology (Figure 1h) and DAPI staining (Figure 1g), whereas, in the case of the truncated gene, the morphology of cells was abnormal (Figure 1, j and l) and DAPI staining still showed chromatin fragmentation (Figure 1, i and k), indicating that the fluorescent DNA fragments observed had nuclear and not mitochondrial origin.

To have a closer look at these nuclear apoptotic effects, we undertook electron microscopy observations. As already detected by DAPI staining, MCY4 cells expressing the truncated KlLsm4p showed chromatin condensation and margination (Figure 2, arrows in b and c) along the inner part of the nuclear envelope, typical of cells undergoing apoptosis and, in some cases, we also observed nuclear fragments (Figure 2d). On the contrary, normal nuclear and cellular morphology was observed when we used as a control the MCY4 strain transformed with the entire *KlLSM4* gene (Figure 2a) or expressing the endogenous *LSM4* gene (our unpublished data).

DNA fragmentation is another hallmark of apoptosis and this phenomenon can be detected in situ by TUNEL staining, which reveals free 3′-OH ends originated by DNA breaks (Gavrieli *et al.*, 1992).

The TUNEL test performed in cells expressing the wild-type *LSM4* and the *KlLSM4* genes (Figure 3, a and b) showed no or only slightly stained nuclei, whereas cells expressing the truncated form of the protein showed an intense staining, indicating that most of cells contained DNA strand breaks (Figure 3c).

Electrophoretic analysis of isolated chromosomal DNA from exponential and stationary phase cultures did not revealed the DNA ladder that has been observed in most, but not all, apoptotic systems (Cornillon *et al.*, 1994) (our unpublished data). This observation, also reported by other authors studying yeast apoptosis, can be explained by the fact that *S. cerevisiae* chromatin structure has short or no linker DNA between nucleosomes (Madeo *et al.*, 1999).

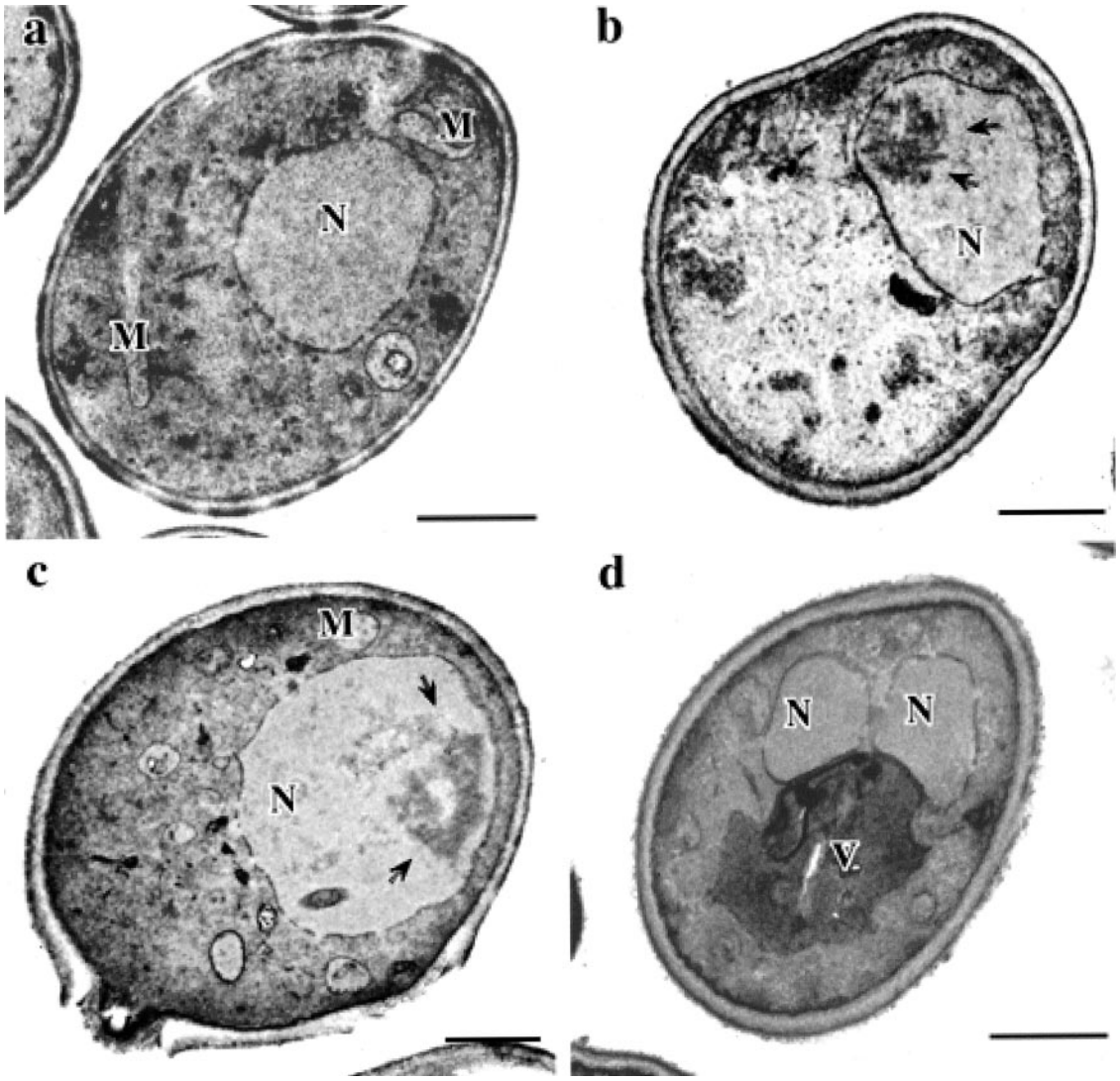

Figure 2. Electron micrographs of strain MCY4-containing plasmids pRS416/*KlLSM4* (a) and pRS416/*Kllsm4Δ1* (b–d) grown on YPD. The arrows indicate the condensed and marginated chromatin. N, nucleus; M, mitochondria; V, vacuole. Bar, 1 μm.

Cells Expressing the Truncated Form Kllsm4Δ1p *Accumulate ROS*

The accumulation of ROS is a key event in triggering apoptosis (Madeo *et al*., 1999). ROS accumulate in yeast cells after oxidizing treatment such as exposure to hydrogen peroxide and in senescent cells (Laun *et al*., 2001). MCY4 strains expressing the entire *KlLSM4* gene or its truncated form were tested for the production of ROS during growth by incubation with dihydrorhodamine 123. This substance accumulates in the cell and is oxidized to the fluorescent chromophore rhodamine 123 by ROS (Schulz *et al*., 1996)

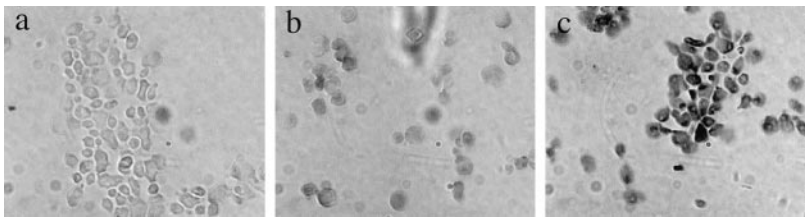

Figure 3. DNA strand breakage detection. To express the endogenous LSM4p, MCY4 cells were grown exponentially on galactose (a), whereas MCY4 cells containing plasmids pRS416/*KlLSM4* (b) and pRS416/*KllsmΔ1* (c) were grown on YPD. After cell fixing and digestion of cell walls, strands breaks in DNA were detected according to the TUNEL protocol.

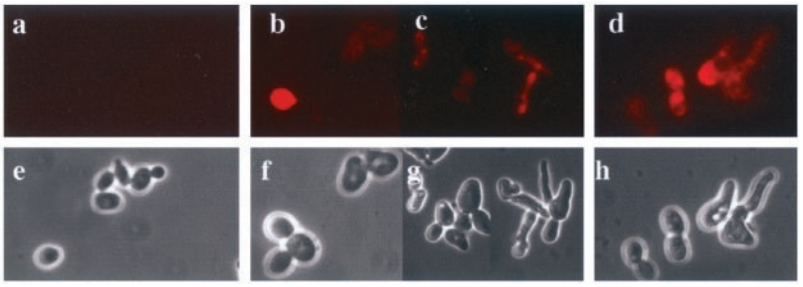

Figure 4. MCY4 expressing *Kllsm4Δ1* accumulates ROS. Cell fluorescence (a–d) and phase contrast displays of cells (e–h) after 2 h incubation with dihydrorhodamine 123 are shown. Wild-type control (a–e), MCY4/*Kllsm4Δ1* (b, c, f, and g) grown in glucose and after 2 h incubation with 4 mM H_2O_2 (d and h).

Substantially, <1% of cells expressing the entire *KlLSM4* gene showed a marginal fluorescence as described previously (Madeo *et al.*, 1999), whereas ~40% of cells expressing the truncated form of the gene showed an intense intracellular staining with DHR (Figure 4, b and c).

H_2O_2 treatment still increased ROS production in both strains in that 80% of cells transformed either with the entire or the truncated *KlLSM4* gene (Figure 4d) became highly fluorescent.

mRNA Stability Increased in the Presence of the Truncated Form of KlLSM4 Allele

In *S. cerevisiae*, the role of Lsm4p has been associated with mRNA decapping, an important step in mRNA degradation, and pre-mRNA splicing (He and Parker, 2000).

To investigate whether the mRNA degradation was altered in the presence of the truncated form of KlLsm4p, we followed the stability of the heat shock gene *SSA4*. This gene is induced at the transcriptional level by high temperatures and, after backshift to the normal temperature, its transcription is shut off and the amount of the mRNA decreases to the basal level within 2 h (Boorstein and Craig, 1990). We looked at the *SSA4* mRNA levels in strains expressing the *LSM4* endogenous gene, the *KlLSM4* gene, and its truncated form Kllsm4Δ1.

As shown in Figure 5A, incubating wild-type or *lsm4*/p*KlLSM4* cells for 15 min at 45°C induced the *SSA4* gene (lanes 2, 8, and 14); the transcript level reached a maximum 15 min after the backshift to 24°C (lanes 3, 9, and 15) and then declined back to the basal levels by 2 h. mRNA degradation followed very similar kinetics for strains expressing *LSM4* (lanes 3–6) and *KlLSM4* (lanes 9–12). In the case of Kllsm4Δ1p, we observed a significant increase in mRNA stability (lanes 16 and 17) in that only a slight decrease in *SSA4* mRNA was seen after shift back to 24°C (lane 18). mRNA stabilization also occurs in *K. lactis* cells expressing Kllsm4Δ1p, which showed an increased stability of the ethanol-repressible gene *KlADH3* (Mazzoni, Mancini, Madeo, and Falcone; unpublished data).

As already mentioned, in *S. cerevisiae* the depletion of Lsm4p causes defects in the excision of introns from pre-mRNAs, indicating a role for this protein also in mRNA maturation (Cooper *et al.*, 1995). To investigate a possible effect of Kllsm4Δ1p on pre-mRNA splicing, total RNA was extracted from strains MCY4 (grown on galactose) and MCY4/*KlLSM4* and MCY4/*Kllsm4Δ1* (grown on glucose), and splicing efficiency was determined by probing Northern blots with the yeast *SNR17* gene (U3 snoRNA; see MATERIALS AND METHODS).

After prolonged autoradiographic exposure, as shown in Figure 5B, we could detect in cells expressing Kllsm4Δ1p the presence of the U3 precursor, which accumulated at a lower level than that observed in cells not expressing LSM4 (Mayes *et al.*, 1999).

S. cerevisiae Strains Lacking Specific Components of the mRNA Decay Machinery Show Apoptotic Phenotypes

We wanted to verify whether the apoptotic markers observed in strains expressing the truncated Lsm4p also occurred in strains lacking other components of the Sm-like complex involved in mRNA decay.

Lsm1p is a specific component of the Lsm1p-7p complex and is not present in the Lsm2p-8p complex involved in mRNA splicing (Bouveret *et al.*, 2000; Tharun *et al.*, 2000). As

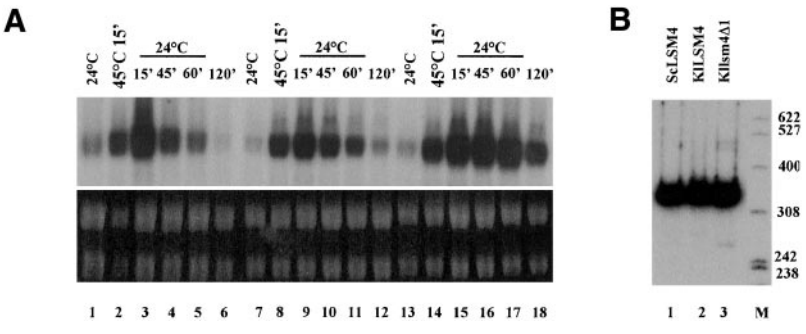

Figure 5. MCY4 expressing *Kllsm4Δ1* shows an increased mRNA stability. Strain MCY4 was grown on YP-galactose to induce the GAL1-*LSM4* gene (lanes 1–6), whereas MCY4/*KlLSM4* (lanes 7–12) and MCY4/*Kllsm4Δ1* (lanes 13–18) were grown on YPD media. (A) Total RNAs were prepared from cells grown overnight at 24°C. After 15 h of incubation at 45°C, cultures were backshifted at 24°C and RNA samples were prepared after 15 min, 45 min, 60 min, and 120 min and probed with the *SSA4* gene. (B) Total RNAs prepared from overnight cultures and probed with the *SNR17* gene (see MATERIALS AND METHODS). M, RNA molecular weight marker.

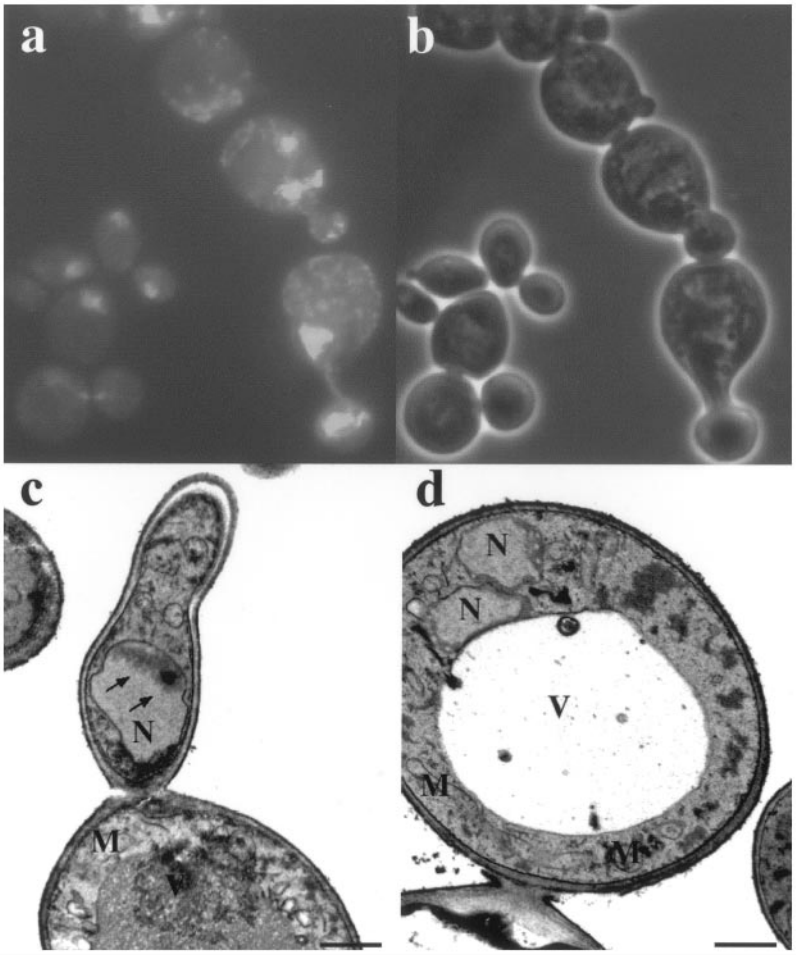

Figure 6. DAPI staining (a and b) and EM analysis (c and d) *of S. cerevisiae* mutant strain *lsm1*. N, nucleus; M, mitochondria; V, vacuole. Bar, 1 μm.

can be seen in Figure 6, DAPI staining and electron microscopy (EM) analysis of cells lacking Lsm1p showed evident chromatin fragmentation (Figure 6a), chromatin condensation along the nuclear membrane (Figure 6c) and the presence of multiple nuclei (Figure 6d), indicating that apoptosis occurred also in this *lsm* mutant.

We also investigated the onset of apoptosis in strains lacking other factors that are known to interact with Lsm1p-7p: Dcp1p and Dcp2p (Beelman *et al.*, 1996; LaGrandeur and Parker, 1998; Dunckley and Parker, 1999), which are two subunits of the decapping holoenzyme (Roy Parker, personal communication); Dhh1p, which is involved in decapping (Coller *et al.*, 2001; Fischer and Weis, 2002); and Pat1p. The Lsm1p-7p/Pat1p complex, independently of its role in promoting decapping (Hatfield *et al.*, 1996; Bonnerot *et al.*, 2000; Bouveret *et al.*, 2000; Tharun *et al.*, 2000), is also involved in the protection of the 3′ end of the mRNA from trimming (He and Parker, 2001).

Strains lacking each of these factors were analyzed by DAPI, TUNEL, and DHR staining. As shown in Figure 7, *dcp1* and *dcp2* mutants showed evident DNA damages as well as increased ROS production, and these phenotypes could be also detected, although at a lower level, in *dhh1* and *pat1* mutants.

EM analysis confirmed that apoptotic phenotypes were more severe in mutants *dcp1* and *dcp2* (Figure 8, a and b) compared with mutants *dhh1* and *pat1* (Figure 8, c and d).

DISCUSSION

In a previous work, we reported the construction of a viable *K. lactis* mutant carrying a truncated form of the essential gene *KlLSM4* and we showed that this mutated allele could also rescue viability in an *S. cerevisiae* strain deprived of its endogenous Lsm4p. Nevertheless, in both yeasts *K. lactis* and *S. cerevisiae*, cells expressing this mutated protein showed a consistent loss of viability as soon as they reached the stationary phase (Mazzoni and Falcone, 2001).

In this work, we report that yeast cells expressing this truncated protein show cytological markers of apoptosis such as nuclei fragmentation and chromatin condensation.

The question as to whether mitochondria are necessary for mammalian apoptosis is very much under discussion (Reed and Green, 2002). A similar debate concerning the role of mitochondria in yeast apoptosis is also ongoing, with some authors suggesting an important role for mitochondria, whereas others report that this process does not necessarily

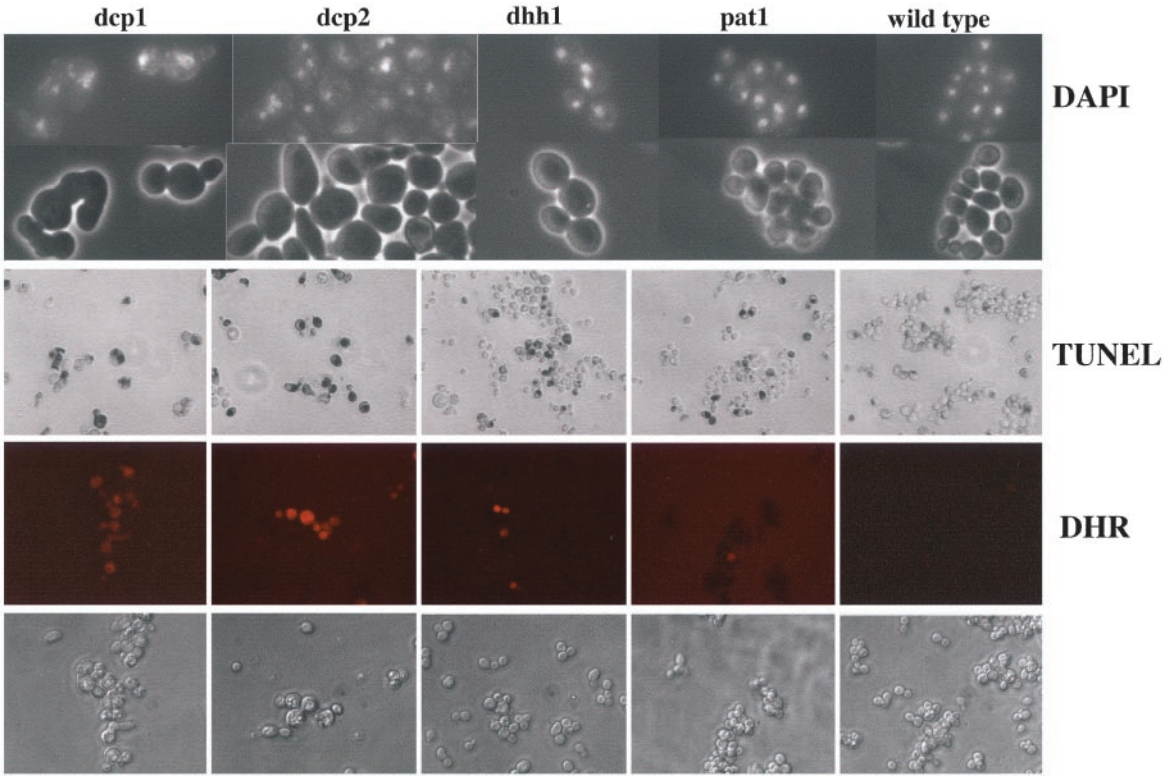

Figure 7. DAPI staining and its phase contrast, TUNEL test, DHR staining of *S. cerevisiae* mutants *dcp1*, *dcp2*, *dhh1*, and *pat1* (see text and MATERIALS AND METHODS for details).

require mitochondrial function (Gross *et al.*, 2000; Kissova *et al.*, 2000). We observed that chromatin fragmentation occurred in KlLsm4Δ1 [*rho*°] cells lacking mitochondrial DNA, indicating that functional mitochondria are not required for this particular pathway of yeast apoptosis.

Almost all scenarios described about apoptosis in yeast so far were somehow connected with oxygen radicals (Madeo *et al.*, 1999; Laun *et al.*,2001). Also, in our case, dihydrorhodamine staining revealed that a high percentage of cells expressing the truncated KlLsm4p accumulated oxidizing molecules (ROS) in the absence of external oxidative stress.

What has been clearly demonstrated in yeast is that Lsm4p plays an important role in mRNA decapping and splicing when assembled in different complexes with other proteins of the Lsm family.

The analysis of cells expressing *Kllsm4Δ1* showed a slight defect in RNA splicing, whereas RNA degradation was significantly delayed in both *S. cerevisiae* and *K. lactis* (our unpublished data).

The onset of apoptosis is not a specific case due to the truncation of Lsm4p in that we demonstrated that the absence of other components of the RNA decay machinery result in the same phenotypes. In fact, very strong effects have also been observed in strains lacking Lsm6p (our unpublished data) and Lsm1p, a protein that differently from Lsm2p-Lsm7p, which are involved both in splicing and mRNA decay, is specific for the latter activity.

Very interestingly, among the other factors that interact with the Lsm1p-7p complex, we found that in the absence of the decapping mRNA enzymes, Dcp1p and Dcp2p, cells show the same phenotypes observed in the absence of Lsm proteins, indicating the decapping is a crucial step in triggering apoptosis.

In the case of mutants in *DHH1*, the gene encoding a DEAD-box RNA helicase involved in mRNA decapping together with Lsm1p-7p (Coller *et al.*, 2001; Fischer and Weis, 2002), the apoptotic markers resulted less evident compared with *dcp*1, *dcp2*, and *lsm* mutants.

A particular case has been observed in the *pat1* mutant that showed a very similar picture to the wild-type strain for DAPI and DHR staining, whereas a significant percentage of cell resulted positive to TUNEL test and EM observation. Although we cannot exclude a relationship between the mRNA 3′ protection and apoptosis, the less severe effects observed in the *pat1* mutant, which has defects in both decapping and trimming protection, together with the existence of a normal mRNA 3′ protection in the *dcp1* mutant (He and Parker 2001), indicated that defects in the latter process does not have a strong effect as observed for decapping.

It is well known that mammalian genes involved in cell cycle and apoptosis are tightly regulated at the transcriptional and posttranscriptional level (Guhaniyogi and Brewer, 2001;. Lam *et al.*, 2001). Our results in yeast suggest that the stabilization of mRNAs could lead to the abnormal production of some protein(s), which in turn trigger apoptosis. Related to this, it is interesting to remark that Dhh1p and Pat1p play also a role in mRNA translation and that their absence result in a less efficient

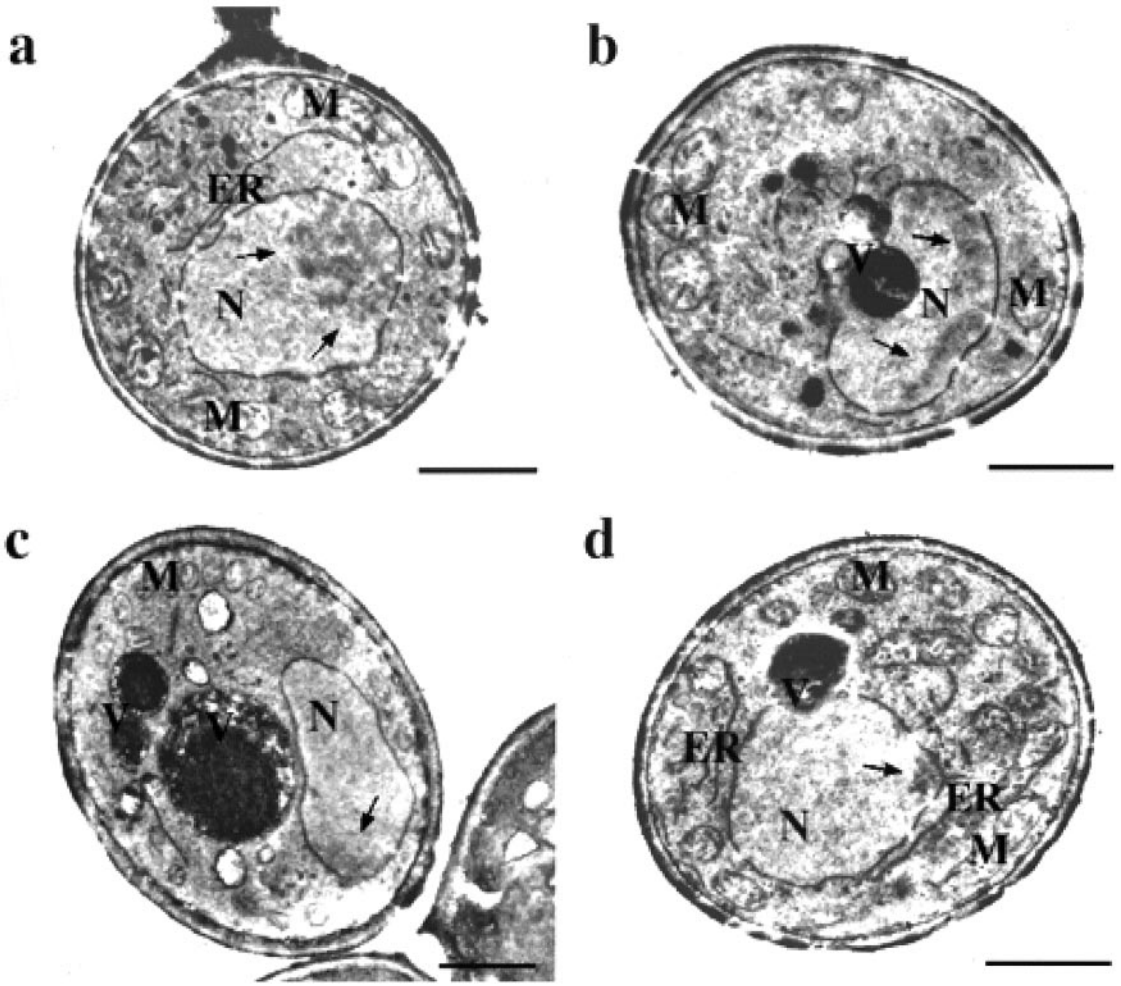

Figure 8. Electron micrographs of strains *dcp1* (a), *dcp2* (b), *dhh1* (c), and *pat1* (d). The arrows indicate the condensed and marginated chromatin. N, nucleus; M, mitochondria; V, vacuole; ER, endoplasmic reticulum. Bar, 1 μm.

protein production (Coller *et al.*, 2001; Wyers *et al.*, 2000; Tharun and Parker, 2001).

One can hypothesize that in these mutants the increased stability of transcription products could be counteracted by a less efficient translation of the accumulated mRNAs, which result in a less severe apoptotic phenotypes. Alternatively, the defects in translation would retard the synthesis of an apoptosis-promoting factor, as suggested by the resistance of H_2O_2-treated cells to entering in apoptosis in the presence of the protein synthesis inhibitor cycloheximide (Madeo *et al.*, 1999; Ligr *et al.*, 2001). Further work has to be done to understand whether the stabilization of total mRNAs or some mRNAs encoding specific proteins is the signal that trigger cellular apoptosis.

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