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The C-terminus of the yeast Lsm4p is required for the association to P-bodies

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Abstract **We previously reported that *Saccharomyces cerevisiae* mutants in mRNA decapping and mutants expressing a truncated form of the *KlLSM4* gene, showed premature senescence and apoptotic phenotypes.**

Here, we show that this truncated protein is dispersed in the cytoplasm and does not assemble to P-bodies. As reported in decapping mutants, we observed an increase in the number of P-bodies suggesting that the truncation of the protein impairs this process. The number of P-bodies also increases after oxidative stress and is not dependent on the meta-caspase gene *YCA1*, placing this phenomenon upstream to the onset of apoptosis.

Keywords: Yeast; Apoptosis; P-body; Oxidative stress; LSM4

1. Introduction

In eukaryotes, the decay of mRNAs commonly starts with the deadenylation step that consists in the removal of the poly(A) tail at the 3′ end of mRNAs [1]. In the yeast *Saccharomyces cerevisiae*, the removal of the poly(A) tail triggers the cleavage of the structure that caps 5′mRNAs, a process called "decapping", which is followed by the degradation from 5′ to 3′ of mRNAs by an exonuclease. Messenger RNAs can be also degraded in the opposite direction by the exosome, a protein complex containing nucleases that works in the 3′ to 5′ direction. The balance between these two pathways varies among mRNAs and organisms [2].

The proteins in the decapping decay pathway localize to specific sites in the cytoplasm of *S. cerevisiae* [3], called processing bodies (P-bodies), which have been also found in mammalian cells [4,5]. These foci contain decapping enzymes (Dcp1p and Dcp2p), the 5′ to 3′ mRNA exonuclease (Xrn1p), proteins that bind to the mRNA after deadenylation (Lsm1–7 proteins), and two other proteins that enhance decapping (Dhh1p and Patlp) [3]. The number of P-bodies is quite low, just two or three per cell, but mutations that eliminate decapping (*dcp1*, *lsm1*, etc.) or the Xrn1p exonuclease increase the size and number of these foci, suggesting that cells provide more sites, although less efficient, for mRNA degradation [3]. In our laboratory, we found that a truncated form of the *Kluyveromyces lactis* essential gene *KlLSM4* (*Kllsm4*Δ1), a component of the Lsm1–7 complex [6,7], supported the growth of a *S. cerevisiae* mutant not expressing the endogenous *LSM4* gene [8]. We demonstrated that stationary phase cells expressing Kllsm4Δ1p, as well other mutants of the yeast mRNA-decapping pathway lacking Lsm1p, Lsm6p Dcp1 and Dcp2, showed increased mRNA stability and entered apoptosis [9,10]. We also showed that this kind of cellular death, was dependent on the yeast meta-caspase encoded by the *YCA1* gene [11].

Lsm proteins, conserved through evolution from Archaebacteria to humans, share a common amino acid motif, called the Sm domain, which is also present in the Sm protein family [12–14]. These conserved motifs, about 70–80 amino acids in length, are localized at the N-terminal region of the proteins and are followed by variable C terminal regions [12,15].

In Kllsm4Δ1p, the two conserved Sm-like domains are still present and we showed that in cells expressing this truncated protein the degradation of mRNAs was delayed while the pre-mRNA splicing process was unaffected [10]. It has been previously reported that mutations in the Sm domains abolished accumulation of YFP-hLSm4 in cytoplasmic foci [5].

Here, we report that the truncated protein Kllsm4Δ1p, still containing the Sm motifs, fails to associate to P-bodies suggesting a role of the C-terminal missing region in this process. Although unable to associate to P-bodies, Kllsm4Δ1p still localized in the nucleus and, as other mutants of the decapping pathway, strains expressing Kllsm4Δ1p showed an increased number of P-bodies. This phenomenon was also observed after H_2O_2 treatment, suggesting that the presence of ROS might induce the formation of P-bodies and, subsequently, trigger cell death signals. The increase in P-bodies might be an early response to the oxidative stress insult, a phenomenon that seems not dependent on the caspase activity in that H_2O_2 still stimulated the proliferation of P-bodies in *yca1*Δ null mutants.

2. Materials and methods

2.1. Yeast strains, growth conditions and plasmids

S. cerevisiae strains used in this study are listed in Table 1.

Cells were grown at 28 °C in YP (1% yeast extract, 2% peptone) supplemented with 2% glucose (YPD) and 2% galactose or in S.D. (yeast nitrogen base without amino acids) with auxotrophic requirements added as needed. Solid media were supplemented with 2% Bactoagar (Difco, Detroit, MI, USA).

The pKlLSM4-GFP and pDHH1-GFP plasmids were obtained by inserting a EcoRI/SalI fragment carrying the *KlLSM4* and *DHH1* ORFs, respectively, previously amplified by PCR (primers in Table 2), into the EcoRI/SalI sites of pUG23 vector (U. Guldener and J. H. Hegemann, Institut fur Mikrobiologie, Heinrich Heine University,

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Table 1
Yeast strains used in this study

Strain	Genotype	Reference
MCY4	Matα, *ade1-101, his3-Δ1, trp1-289, ura3, LEU2-GAL1-SDB23*	[6]
MCY4/*KlLSM4*	Matα, *ade1-101*, *his3- Δ1*, *trp1-289*, *ura3*, *LEU2-GAL1-SDB23*, pRS416/*KlLSM4*	[8]
CML48	MCY4, pUG23/*KlLSM4-GFP*	This work
CML49	MCY4, pUG23/*Kllsm4Δ1-GFP*	This work
CML50	MCY4, pUG23	This work
CML54	MCY4, pRS416/*KlLSM4*, pUG23/*DHH1-GFP*	This work
MCY4/*Kllsm4Δ::kanMX4*	MCY4, pRS416/*Kllsm4Δ::kanMX4*	[8]
CML51	MCY4, pRS416/*Kllsm4Δ::kanMX4*, pUG23/*DHH1-GFP*	This work
CML39-8D	Mata, *ade1-101, his3-Δ1, trp1-289, ura3, LEU2-GAL1-SDB23*, yor197w::KanMX4, pRS313/*Kllsm4Δ1*	[11]
CML52	Mata, *ade1-101, his3-Δ1, trp1-289, ura3, LEU2-GAL1-SDB23*, yor197w::KanMX4, pRS416/*Kllsm4Δ::kanMX4*, pUG23/*DHH1-GFP*	This work
CML39-11A	Mata, *ade1-101, his3-Δ1, trp1-289, ura3, leu2*	[11]
CML39-9A	Mata, *ade1-101, his3-Δ1, trp1-289, ura3, leu2,* yor197w::KanMX4	[11]
BMA38	Matα, *ura3-1, leu2-3, -112, ade2-1, can1-100, his3-11, -15, trp1Δ1*	[13]
AEMY24 (*lsm1*)	Matα, *ura3-1, leu2-3, -112, ade2-1, can1-100, his3-11, -15, trp1Δ1, lsm1Δ::TRP1*	[13]

Table 2
Oligonucleotides used in this work

KlLSM4GFPfor	5′CCCCCGAATTCATGCTTCCATTATATTTATTGAC 3′
KlLSM4GFPrev	5′CCCCCGTCGACAAATTGTGGAGTTTGATTACTATTG 3′
DHH1GFPfor	5′CCCCCGGATCCATGGGTTCCATCAATAATAACTTC 3′
DHH1GFPrev	5′CCCCCGTCGACATACTGGGGTTGTGACTGACCAG 3′

Dusseldorf, Germany; unpublished results; plasmid information available online at http://mips.gsf.de/proj/yeast/info/tools/hegemann/gfp.html). Plasmid pKllsm4Δ1-GFP was obtained by cloning the 216 bp BamHI/EcoRV fragment from pKlLSM4-GFP, containing the first 72 amino acids of the KlLsm4p, into the BamHI/EcoRV sites of pUG23 vector. Yeast transformation was performed by electroporation [16].

The correct transcription of the fused genes was verified by hybridization to probes corresponding to *GFP* and *Kllsm4*Δ1.

Proteins of the expected sizes were detected by western analysis using the αGFP antibody (Boehringer–Mannheim, Germany) directed against the GFP.

Proteins were prepared according to the TCA method, as previously described [17].Western blot and immunodetection by enhanced chemoluminescence (ECL; SuperSignal, System, Pierce) was performed following standard methods [16].

2.1.1. Microscopy fluorescence. For the in vivo staining of nuclei, cells were treated with the DAPI dye at the final concentration of 2.5 μg/ml and protected from light before microscopic observation [18]. For image acquisition, we used an Axioskop 2 fluorescence microscope (Carl Zeiss, Jena Germany), a digital camera (micro CCD, model RGB-MS-C CRI Inc., Boston, MA) and CCD software (version 3.11, Apogee Instruments Inc., Auburn, CA).

3. Results and discussion

3.1. The KlLsm4p-GFP and Kllsm4Δ1p-GFP fusion proteins are functional

In previous works, we described that the *Kllsm4*Δ1, a truncated from of the *K. lactis* essential gene *KlLSM4*, could compensate the absence of orthologous *LSM4* gene of *S. cerevisiae* [8] but, in the absence of the C-terminus of the protein, cells underwent apoptosis through a caspase-dependent pathway [11]. To investigate the localization of this protein within the cell, the complete and the truncated forms of the *KlLSM4* gene were fused to *GFP* and transformed into MCY4, the *S. cerevisiae* strain harbouring the own *LSM4* (*SDB23*) gene under the control of the inducible *GAL1* promoter [6]. The growth of this strain is therefore galactose-dependent and does not occur in the presence of glucose. As shown in Fig. 1, panel A, in contrast to cells expressing *GFP* alone (CML50) or untransformed cells (MCY4), both strains expressing the individual fusions (CML48 and CML49) could grow in glucose, indicating that both proteins were functional. The correct transcription and translation of the fusions were also confirmed by northern and Western analysis (see Section 2).

We previously reported that cells expressing *Kllsm4Δ1* loose viability during stationary phase very rapidly and are more sensitive to oxidative stress [11]. To verify if the fusions to GFP altered the properties of the KlLsm4 proteins, we measured the viability of the CML48 and CML49 strains during normal growth and after treatment with H_2O_2. As shown in Fig. 1B and C, CML49 showed reduced viability compared to CML48 both in stationary phase and after H_2O_2 treatments, confirming what already observed with the same proteins not fused to GFP.

3.2. Cellular localization of KlLsm4p-GFP and Kllsm4Δ1p-GFP

It has been reported that the components of the Lsm1–7 complex, which function in mRNA degradation, essentially localize in cytoplasmic foci named P-bodies [3–5]. The presence of P-bodies inside the cell is directly related to the mRNA flux into these foci. In fact, the treatment with cycloheximide rapidly inhibits decapping, presumably by trapping mRNAs on polysomes [19], and caused a loss of P-bodies within 10 min [3]. We wanted to study the cellular localization of the KlLSM4-GFP proteins and, as a control, we constructed a strain expressing the DEAD-box helicase Dhh1p, a protein