RESEARCH ARTICLE

Analysis of the rpn11-m1 proteasomal mutant reveals connection between cell cycle and mitochondrial biogenesis

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Received 22 February 2010; revised 20 July 2010; accepted 22 September 2010. Final version published online 9 November 2010.

DOI:10.1111/j.1567-1364.2010.00690.x

Editor: Monique Bolotin-Fukuhara

Abstract

The proteasomal lid subunit Rpn11 is essential for maintaining a correct cell cycle and mitochondrial morphology in Saccharomyces cerevisiae. In this paper, we show that the rpn11-m1 mutant has a peculiar cell cycle defect reminiscent of mutants defective in the FEAR pathway that delay the release of the Cdc14 protein phosphatase from the nucleolus. We analyzed the rpn11-m1 phenotypes and found that overexpression of Cdc14 suppresses all the rpn11-m1 defects, including the mitochondrial ones. Suppression by Cdc14 of the rpn11-m1 mitochondrial morphology defect reveals an uncharacterized connection between mitochondrial and cell cycle events. Interestingly, the overexpression of Cdc14 also partially restores the tubular network in an Dmmm2 strain, which lacks a mitochondrial protein belonging to the complex necessary to anchor the mitochondrion to the actin cytoskeleton. Altogether our findings indicate, for the first time, a cross-talk between the cell cycle and mitochondrial morphology.

Introduction

The proteasome system is essential for the degradation of short-lived proteins that are involved in cell cycle regulation, DNA repair, apoptosis, regulation of metabolism, signal transduction, besides the essential function of degradation of misfolded or damaged proteins (Hershko & Ciechanover, 1998; Schwartz & Ciechanover, 1999). The 26S proteasome is a multicatalytic complex formed by the 20S core particle and the 19S regulatory particle (RP). The RP can be divided in two subcomplexes, the base and the lid (Glickman et al., 1998; Leggett et al., 2002; Funakoshi et al., 2004; Sone et al., 2004). The Rpn11 protein is the proteasomal deubiquitinating enzyme. This protein contains at least two domains performing complex functions: the deubiquitinating motif is located in the N-terminal part (Verma et al., 2002), while the C-terminal part is responsible for the stabilization of the lid (Rinaldi et al., 2004), for cell cycle progression and for maintaining a correct mitochondrial morphology (Rinaldi et al., 1998, 2008).

Our previous studies, using a truncated version of Rpn11 (Rpn11-m1, lacking the last 31 amino acids), suggested that the cell cycle and the mitochondrial phenotypes could be separated and that the N- and C-terminal domains seemed to perform independent functions; this result was also supported by the localization of Rpn11, which was found to be associated also with the mitochondrial membranes (Rinaldi et al., 2002, 2008). However, we were unable to select revertants from the rpn11-m1 mutant showing a wild-type tubular mitochondrial network but still displaying cell cycle defects, indicating that to re-establish a wild-type mitochondrial phenotype in the mutant it was first necessary to correct the cell cycle defect. This point suggested a coordination between these two essential events, but up to now, no evidence for this had been found.

Here, we study the involvement of some proteins essential for cell cycle progression in the cell cycle defects of rpn11-m1. This holds in particular for the Cdc14 phosphatase, which plays an essential role in the progression from chromosome segregation to cytokinesis. Cdc14 is a protein phosphatase likely to be the most downstream element in the mitotic exit because it can act directly on cell cycle regulators to promote exit from mitosis. This phosphatase is
sequestered into the nucleolus throughout most of the cell cycle and is released in two waves under the action of the FEAR (Cdc fourteen early anaphase release) and MEN (mitotic exit network) pathways, which control the exit from mitosis (Geymonat et al., 2002; Pereira et al., 2002; Stegmeier et al., 2002; D’Amours & Amon, 2004a; Seshan & Amon, 2004). When liberated from the nucleolus in anaphase, Cdc14 acts on key substrates to promote a decrease in CKK/B-cyclin activity and mitotic exit. Recently, it has been shown that the phosphatase activity of Cdc14 is required for RNA polymerase I (Pol I) inhibition and that Pol I transcription interfered with ribosomal DNA (rDNA) condensation (Clemente-Blanco et al., 2009). The present results show a central role of the phosphatase Cdc14 not only in correcting the cell cycle phenotype of rpn11-m1 but also in suppressing the mitochondrial morphology defect.

We have previously demonstrated that the Rpn11 protein is involved in the maintenance of the wild-type mitochondrial network, in particular acting on the tubulation and fission apparatus (Rinaldi et al., 2008; Hofmann et al., 2009). The tubulation apparatus, also called the mitochore complex (for review, see Okamoto & Shaw, 2005; Boldogh & Pon, 2006), is formed by Mdm10, Mdm12, Mmm1 and Mmm2 proteins. Very recently, all these proteins, together with Mmm2, were found to be part of the ERMES complex, whose function is to participate in the endoplasmic reticulum–mitochondria contacts for calcium and phospholipid exchange (Kormann et al., 2009). Previously, we showed that the absence of the Mmm2 protein results in a semi-lethal phenotype when associated to the rpn11-m1 allele. Here, we show that Cdc14 overexpression alleviates (in addition to rpn11-m1) the mitochondrial morphology defect of a Δm11m2 strain, revealing an important coordination between exit from mitosis and mitochondrial biogenesis.

Materials and methods

Strains, plasmids and media

The strains used in this study were all in W303 genetic context (his3-11, ade2-1, leu2-3,-112, ura3-1, trp1-1, can1-100). The plasmids used were: pRS316-GAR1-GFP, kindly provided by Marlene Oeffinger; pAFS125-TUB1-GFP-TUB1 (integrative plasmid); pRS306-CDC14-GFP-URA3 (multicopy plasmid, to integrate into URA3 locus the plasmid was digested with XcmI); pRS315-CDC14 (centromeric plasmid), kindly provided by Elmar Schiebel; and the mitoGFP plasmid pYX232 (Westermann & Neupert, 2000). YPD (1% Bacto peptone, 1% yeast extract and 2% glucose), YPG (1% Bacto peptone, 1% yeast extract and 2% glycerol), YPGal (1% Bacto peptone, 1% yeast extract and 2% galactose) were used as yeast culture-rich media. WO (0.17% yeast nitrogen base, 0.5% ammonium sulfate and 2% glucose) was used as minimal medium. All media were supplemented with 2.3% Bacto agar (Difco) for solid media and WO was supplemented with the appropriate nutritional requirements according to the phenotype of the strains. The culture media for sporulation were preporulation media (0.5% yeast extract, 2% Bacto peptone, 2% potassium acetate) and 1% potassium acetate. Yeast cultures were grown at 24 or 28 °C; the nonpermissive temperature was 36 °C.

Activation of CDC14 gene under the GAL1-10 promoter

The strains (W303 genetic context) used in this study were generated by mating, sporulation and tetrad dissection. To produce the rpn11-m1 mutant completely deleted of the rDNA region (rpn11-m1ΔrDNA), the rpn11-m1 mutant was crossed with the ΔrDNA strain; the growth of this strain is supported by the presence of a plasmid containing a rDNA unit under a Pol II GAL promoter (Wai et al., 2000). After sporulation of the diploid strain and tetrad analysis, we selected the rpn11-m1 mutant strain with the deletion of the chromosomal rDNA. The strains (rpn11-m1 and Δm11m2) with the integrated cassette GAL-CDC14 were generated by crossing the WT-GAL-CDC14 strain (Visintin et al., 1998) and following the URA3 marker after tetrad dissection. The WT-TUB1-GFP and rpn11-m1-TUB1-GFP strains were produced by integration of the integrative plasmid pAFS125-TUB1-GFP-TUB1. The rpn11-m1-NUP49-GFP strain was produced by mating the WT-NUP49-GFP (Invitrogen) with rpn11-m1; the recombinant strain was selected after tetrad dissection. Cells were cultured at 28 °C to exponential phase, half of the cultures were shifted at the nonpermissive temperature for 5 h, and then cells were observed in the fluorescence microscope. The CDC14-GFP fusion was integrated in the URA3 locus, transforming the wild-type, rpn11-m1 and Δm11m2 strains with the pRS306-CDC14-GFP-URA3 plasmid linearized at the XcmI restriction site.
network, the cells after activation were cultured for 1 h on YPD and then observed by fluorescence microscopy.

**Western blot analysis**

Yeast strains were grown to OD$_{600 \text{nm}}$ of 1.0 at 28 °C in YPD medium. After activation in YPGal medium, the cultures were collected and harvested, and protein extraction was performed using glass beads in ice-cold lysis buffer according to Alonso-Monge et al. (2003). Proteins were resolved on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels, transferred to polyvinylidene fluoride membranes and analyzed by immunoblotting using the polyclonal antibody Cdc14(yE-17) (61 kDa) (Santa Cruz Biotechnology) and with monoclonal antibody 3-phosphoglycerate kinase (45 kDa) (Molecular Probes, Invitrogen, CA). The secondary antibodies were monoclonal anti-mouse immunoglobulin G and anti-goat conjugated with peroxidase (Promega, Milan, Italy). Immunologically active proteins were visualized with the electrochemiluminescence detection system (Amersham, Arlington Heights, IL) according to the manufacturer’s instructions.

**Rho$^-$ production**

To better visualize the nucleus, we produced cells devoid of mtDNA. Cells were grown at the density of $1 \times 10^6$ cells mL$^{-1}$ on YPD medium. The following day, phosphate buffer pH 6.5 at a final concentration of 0.05 M and ethidium bromide at a final concentration 50 μg mL$^{-1}$ were added to 1 mL of culture. The culture was incubated at 28 °C for 24 h. After washing the cells twice in H$_2$O, the cells were plated on an YPD plate and incubated for 2–3 days at 28 °C.

**Microscopy**

The $rpn11$-$m1$ cells cannot be synchronized with the α-factor, so the cultures were grown to stationary phase in solid YPD complete medium and then shifted at 36 °C for 5 h. Cells were fixed and DAPI was added to the final concentration of 2.5 μg mL$^{-1}$ and analyzed by fluorescence microscopy. Filters for green fluorescent protein (GFP) (450/490 nm excitation and 500/550 nm emission) and DAPI (340/380 nm excitation and 450/490 nm emission) were used. The Axioskop 2 fluorescence microscope (Carl Zeiss, Jena, Germany), equipped with a digital camera MicroCCD, was used for image acquisition. Images of Figs 2 and 6 were captured with a DMI20 microscope (Leica, Deerfield, IL) equipped with a CCD camera (Roper Scientific, Tucson, AZ). METAMORPH software (Universal Imaging, West Chester, PA) was used to deconvolute Z-series and treat the images.

![Fig. 1. Visualization of the nuclear envelope and nuclear DNA in $rpn11$-$m1$ proteasomal mutant. WT-NUP49-GFP and $rpn11$-$m1$-NUP49-GFP rho$^-$ strains were grown at 28 °C and then shifted at the nonpermissive temperature of 36 °C for 5 h and stained with DAPI. The GFP signal marks the nuclear envelope, which is anchored in the mother cell in $rpn11$-$m1$ mutant. The white arrow indicates the thread of DNA corresponding to the rDNA.](https://academic.oup.com/femsyr/article-abstract/11/1/60/630872)
Analyses of the rpn11-m1 cell cycle defects

The rpn11-m1 mutant contains a truncated version of the Rpn11 proteasomal subunit, produced by a frameshift in position 276 (Rinaldi et al., 1998). This mutant could not grow at 36°C and after a shift for 5 h to the nonpermissive temperature, half of the cells showed an elongated bud phenotype and a pre-anaphase arrest with unsegregated chromosomes migrated into the bud (called a daughterly phenotype, Ross & Cohen-Fix, 2004). After 3 h, the buds were elongated and 30% of the cells also showed a thread of uncondensed DNA lagging in the nucleus behind the main chromosomal mass (Fig. 3a). We used rho− cells, i.e. cells devoid of mtDNA, to better visualize the nuclear DNA. These rho− cells behaved as rpn11-m1 cells concerning the cell cycle defects observed. The elongated bud phenotype is well described in the literature and is the consequence of cyclin stabilization and the consequent imbalance of apical and isotropic growth (Lew & Reed, 1993), but the thread of DNA has never been reported in yeast mutants showing the elongated bud phenotype. We therefore investigated this phenotype in detail.

The observed thread of DNA in the rpn11-m1 mutant is the rDNA

The rpn11-m1 mutant shows an unusual thread of DNA extending outside the main chromosomal mass (Fig. 3a). As the segregation of rDNA present in the long arm of chromosome XII during mitosis is known to segregate later than the bulk of chromosomal DNA during mitosis (D’Amours et al., 2004b; Sullivan et al., 2004), we wondered whether the lagging DNA observed in the rpn11-m1 mutant could be the rDNA. To demonstrate that the thread of DNA in rpn11-m1 mutant was specifically rDNA and not a general defect in nuclear DNA segregation, we produced the rpn11-m1 mutant completely deleted of rDNA in the chromosome XII (rpn11-m1/DrDNA); the growth of this strain was supported by the presence of a plasmid containing a rDNA unit under the Pol II GAL1 promoter (Wai et al., 2000). This recombinant strain showed the same growth defect as the single mutant rpn11-m1. After a shift for 5 h at the nonpermissive temperature, the rpn11-m1 and the rpn11-m1/DrDNA cultures were stained with DAPI to visualize DNA. We observed that the rpn11-m1/DrDNA strain, which does not contain the chromosomal rDNA units, did not show the thread of DNA (Fig. 3a), indicating that the lagging DNA, present in the rpn11-m1 mutant, is the chromosomal rDNA.

We then wished to visualize the nucleolus assembled around the rDNA. For this purpose, we transformed the wild-type strain and the rpn11-m1 mutant with a plasmid containing the GAR1 gene fused to GFP. Gar1 is a protein localized in the nucleolus and is a well-known nucleolus marker. After a shift at 36°C for 5 h, cells were stained with DAPI. Figure 3b shows that the GFP signal colocalizes with the thread of DNA in the mutant as compared with the wild-type nucleolar localization. We also noted that at 28°C, the rpn11-m1 permissive temperature, the mutant had a normal nucleolus (appearing as a crescent shape within the nucleus, data not shown). These results demonstrate a specific defect in rDNA and nucleolar segregation in the proteasomal mutant rpn11-m1.
The thread of DNA corresponding to rDNA was always behind the chromosomal mass and attached to the mother cell. This observation is consistent with the fact that the rDNA region is segregated later than the main chromosomal mass (D’Amours et al., 2004b; Sullivan et al., 2004). The visualization of the spindle pole body (by transformation of the strains with a plasmid containing the TUB1-GFP construct), revealed that the rpn11-m1 mutant shows 38.5% of cells with the mitotic spindle localized in the daughter cell, with the phenotype absent in the wild-type strain as shown in Table 1.

Thus, the main chromosomal mass is transported into the daughter cell, as both spindles migrate into the bud, but the rDNA, segregating later, still remains attached in the mother cell. This observation is interesting because it suggests that a nuclear polarity could exist during nuclear migration. The nuclear membrane associated with the nucleolus has properties that distinguish it from the membrane surrounding

Table 1. Position of the mitotic spindle during cell cycle as revealed by Tub1-GFP visualization

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<th>% of cells showing the phenotypes presented below (200 cells per strain)</th>
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the rest of the nucleus (Campbell et al., 2006). To visualize how the thread of rDNA present in rpn11-m1 makes contact with the nuclear envelope, we integrated the NUP49-GFP construct in the rpn11-m1 strain (see Materials and methods). Nup49 is a protein of the pore complex localized in the nuclear envelope (Wente et al., 1992). Figure 1 shows that in the rpn11-m1 strain, the unsegregated DNA mass (stained with DAPI, blue) has migrated into the bud, leaving behind a DNA thread (arrow) surrounded by the nuclear envelope (Nup49-GFp, green), which is retained in the mother cell.

Overexpression of the phosphatase CDC14 gene suppresses the rpn11-m1 cell cycle defects

The specific rDNA segregation defect observed in the rpn11-m1 cells was reminiscent of a Cdc14 dysfunction (D’Amours et al., 2004b). If the FEAR pathway is not, or less, active in the rpn11-m1 strain, the Cdc14 phosphatase should be confined to the nucleolus. To look at the localization of Cdc14, we integrated the CDC14-GFP construct (see Materials and methods) in the wild-type strain and in the rpn11-m1 mutant. Whereas at 28 °C the Cdc14-Gfp signals can also be observed spread in the nucleus, in the mutant as in the wild-type strain, at the nonpermissive temperature the Cdc14-Gfp signals in the mutant were always localized in the nucleolus (Fig. 2), suggesting that rpn11-m1 keeps Cdc14 inactive in the nucleolus due to an absence of FEAR activation.

We wondered whether overexpression of Cdc14 could suppress the rpn11-m1 phenotypes described above. For that purpose, we transformed the wild-type and the rpn11-m1 strains with a centromeric or multicopy plasmid containing the CDC14 gene under the control of its own promoter. The cell cycle defect of the mutant strain was suppressed only in the presence of the multicopy plasmid (data not shown). We observed, as described by Visintin et al. (1998), that the cells seem to be blocked in G1 phase; in fact it was demonstrated that overexpression of Cdc14 produces a block in G1 phase and a lethality phenotype (Visintin et al., 1998). To verify that the suppression was not an artifact of cell cycle arrest, we integrated the CDC14 gene in rpn11-m1 under the transcriptional control of the GAL1-10 promoter (resulting strain rpn11-m1-GAL-CDC14, see Materials and methods). Figure 4 shows that the WT-GAL-CDC14 strain, after activation of CDC14 transcription in YPGal medium, contains 10.32 ± 2.63 of cells with elongated buds. A similar percentage was reported by Visintin et al. (1998) and is due to the high levels of Cdc14 into the cell. At the nonpermissive temperature, when Cdc14 was not overexpressed (glucose), the rpn11-m1-GAL-CDC14 cells showed
35.84% ± 2.95 of cells with the cell cycle defects described above, whereas when Cdc14 was overexpressed (galactose), these cell cycle defects were drastically reduced (13.48% ± 1.49). The rpn11-m1 mutant, grown at the non-permissive temperature on glucose or galactose, showed the same percentage of cells with cell cycle defects (36.7% ± 1.58 and 36.08% ± 1.9, respectively), whereas the wild-type strain did not show any cell cycle defects. These results indicate that overexpression of Cdc14 can suppress all the cell cycle defects of the proteasomal mutant.

Overexpression of the phosphatase Cdc14 also suppresses the rpn11-m1 mitochondrial morphology defect

As the main phenotype of rpn11-m1 cells observed at the permissive temperature in glucose is fragmented mitochondria, we wondered whether the mitochondrial morphology defect could also be alleviated by Cdc14 overexpression. The wild-type and rpn11-m1 strains with the integrated GAL-CDC14 fusion (WT-GAL-CDC14 and rpn11-m1-GAL-CDC14 strains) were transformed with a plasmid encoding GFP addressed to the mitochondria. As the mitochondrial defect in rpn11-m1 is already visualized at the permissive temperature, the cultures were split in two, and after 3 h in galactose or in glucose media, cells were observed with the fluorescence microscope. Figure 5 shows that after the activation for 3 h of CDC14 transcription, 50% of the rpn11-m1 cells showed a wild-type mitochondrial tubular network, whereas in the absence of Cdc14 overexpression (glucose) mitochondria were still fragmented. These results indicate that overexpression of Cdc14 can also suppress the mitochondrial defect of the proteasomal mutant in addition to the cell cycle defects.

Overexpression of the phosphatase Cdc14 also suppresses the mitochondrial morphology defect of a mitochondrial tubulation mutant (Δmmm2)

Our previous work showed that the absence MMM2 belonging to the tubulation apparatus (MDM10, MDM12, MMM1 and MMM2, for a review, see Okamoto & Shaw, 2005) associated with the proteasomal mutation rpn11-m1, resulted in a severe growth defect with highly elongated aberrant cells (Rinaldi et al., 2008).

The severe growth defect phenotype of MMM2 deletion in combination with the rpn11-m1 allele suggests a possible
role of Mmm2 and/or the mitochondrial apparatus in coordinating the cell cycle and the mitochondrial morphology, movement and/or mitochondria transmission. rpn11-m1 does not result in growth defect in association with any other deletion of genes involved in maintenance of the mitochondrial morphology (fusion or fission genes, Rinaldi et al., 2008; Hofmann et al., 2009). Moreover, although the mitochondrial function of Rpn11 has not been fully elucidated, our previous results point to an involvement of Rpn11 in the regulation of the tubulation function (Rinaldi et al., 2008). We wondered whether overexpression of Cdc14 could alleviate the mitochondrial collapsed morphology of the Δmmm2 tubulation mutant. Therefore, we overexpressed Cdc14 in Δmmm2 strain in the same conditions used for the rpn11-m1 mutant. Δmmm2 containing the integrated GAL-CDC14 fusion (Δmmm2-GAL-CDC14) was transformed with the mitoGFP plasmid. A correct overproduction of the Cdc14 protein was checked in the strains containing the integrated GAL-CDC14 fusion after galactose induction (Supporting Information, Fig. S1).

Figure 6 showed that after activation for only 3 h of CDC14 transcription, 40% of the Δmmm2 cells contained a wild-type mitochondrial tubular network, indicating that Cdc14 overexpression can also alleviate the mitochondrial defect of a tubulation mutant as observed for rpn11-m1.

Recently, the Mmm2 protein was also shown to be part of the ERMES complex (Kornmann et al., 2009). Therefore, we examined the ability of overexpressed CDC14 to suppress mitochondrial morphology defects caused by loss of MDM10, MDM12 or MMM1. The three single deleted strains, with an integrated GAL-CDC14 construct, were transformed with the mitoGFP plasmid and observed with fluorescence microscopy. Although mild changes in the mitochondrial morphology were observed in the Δmdm10 strain containing the integrated GAL-CDC14 construct after activation of the GAL promoter (Fig. S2), the overexpression of CDC14 did not restore a tubular network in the ERMES mutants as it did in the Δmmm2 strain (Fig. 6). This indicates that the effects of Cdc14 might be specific to Mmm2. Interestingly, overexpression of CDC14 completely suppressed the multibud phenotype of the Δmdm10 strain. This strain has 9% multibuds, whereas when CDC14 is overexpressed, the percentage decreased to 2% ± 0.5, a result similar to the W303 wild-type strain (2% ± 0.4 multibuds; data not shown).

As the deletion of MMM2 in a W303 context resulted in the absence of growth on glucose at 36 °C, suggesting a defect/delay in cell cycle progression, we integrated the CDC14-GFP construct in the Δmmm2 strain to visualize the Cdc14 localization. Figure 2 clearly shows that Cdc14 is kept in the nucleolus in Δmmm2 cells, in agreement with results obtained for the Δmdm10 strain by L. Pon and colleagues (García-Rodríguez et al., 2009). Altogether, our
results suggest a yet uncharacterized interaction between cell cycle progression and the mitochondrial complex.

Discussion

The present results highlight the mechanisms by which the peculiar cell cycle phenotypes of the proteasomal mutant rpn11-m1 are generated. Here, we show that transient overexpression of the Cdc14 phosphatase suppresses all the rpn11-m1 cell cycle defects. This result suggests that, contrary to the general idea that the proteasomal mutants are affected in the degradation of all ubiquitinated proteins, the proteolytic defects of rpn11-m1 might be more specific for individual proteins. Visintin et al. (1998) have demonstrated that overexpression of the CDC14 gene results in the degradation of Pds1, Clb2 and Clb5 proteins. Therefore, we propose that in rpn11-m1, overexpression of Cdc14 might promote specifically the degradation of the substrates that have accumulated, thus abolishing the elongated bud phenotype; moreover, Pds1 degradation is promoted, leading to chromatid separation (see Morgan & Roberts, 2002; Santa-maria et al., 2003). This is consistent with the results showing that in the rpn11-1/m1Δpds1 double mutant the pre-anaphase arrest was alleviated as compared with the rpn11-m1 mutant (data not shown). The hypothesis of a selective protein degradation is also consistent with our previous observation that rpn11-m1 is not sensitive to canavanine (Rinaldi et al., 2004). This compound is an analogue of arginine, which is inserted in the newly synthesized proteins, producing aberrant proteins that became proteasomal substrates. The proteasomal mutants are in general very sensitive to this drug because they are unable to degrade this bulk of proteins. The lack of sensitivity to canavanine of rpn11-m1 suggests that this mutant is not dramatically affected by the degradation of aberrant proteins.

As for nuclear migration into the bud (daughterly phenotype), a similar phenotype was observed for the diploid double mutant cdc28-4/clb5 (Segal et al., 1998). The spindle assembly and its correct orientation require the action of Cdc28/Clb5, as in the cdc28-4/clb5 double mutant the asymmetric behavior of spindle pole bodies (SPBs) is disrupted and both poles interact with the bud cell cortex, allowing the nucleus to migrate into the bud (Segal et al., 2000). The Cdc28/Clb5 kinase activity is necessary for the phosphorylation of Spc110, a component of the SPB, and this phosphorylation is sensitive to the overexpression of Cdc14 (Huisman et al., 2007). As a phosphatase, Cdc14 could reverse Cdc28/Clb5 phosphorylation on Spc110 or in other substrates, leading to control/maintenance of the spindle polarity, and reverse the forces that ‘pull’ the nucleus into the bud. It is possible that in rpn11-m1, Clb5 is stabilized and that the overexpression of Cdc14 leads to Clb5 degradation. One of the functions of Cdc14 is

Fig. 7. Proposed model for coordination between exit from mitosis and mitochondrial morphology. Degradation of Pds1 protein (inhibitor of Esp1) by the proteasome leads to chromatid segregation (anaphase) and FEAR activation. The FEAR pathway promotes the activation of the phosphatase Cdc14 by releasing it from the nucleolus into the nucleus. The active Cdc14 dephosphorylates its targets, promoting the exit from mitosis. In this paper, we show that overexpression of Cdc14 is capable, by a yet unknown mechanism, indicated by ?, of suppressing the mitochondrial morphology defects of the proteasomal mutant rpn11-m1 and also the absence of the Mmm2 mitochondrial protein. The Mmm2 protein is a key protein that allows the maintenance of the mitochondrial network anchored to the actin cytoskeleton and the combination of rpn11-m1 and Dmmt2 alleles results in a severe growth defect phenotype. We propose a coordination between the exit from mitosis and the mitochondrial morphology and movement.
activation of the forces that retain the nucleus in the mother cell during anaphase (D’Amours & Amon, 2004a). Little is known about how the nucleus is retained in the mother cell, so the rpn11-ml1 strain could be an interesting mutant to investigate this point.

Finally, the absence of rDNA condensation in rpn11-ml1 cells could be due to the absence of Cdc14 activation, as in the cdc14-3 mutant, the rDNA region is pulled between the two nuclei (D’Amours et al., 2004b).

We regard as very important the observation that the overexpression of Cdc14 also suppresses the mitochondrial morphology defect of the rpn11-ml1 mutant. Previous analyses of the mitochondrial morphology defects in this proteasomal mutant have indicated that the Rpn11 protein might be involved in the regulation of the mitochondrial tubulation and fission processes, independently of its proteasomal function (Rinaldi et al., 2008; Hofmann et al., 2009). Now we demonstrated that the collapsed mitochondrial morphology of a tubulation mutant (Δmm2) can also be alleviated by overexpressing Cdc14.

We have previously shown that the mitochondrial function of Rpn11 resides in few C-terminal amino acids overlapping a α-helical region, and that the carboxy-terminal region is acting even in trans (Rinaldi et al., 2008). Up to now, we have considered the two cellular events (cell cycle and mitochondrial morphology) independently, but the suppression of the rpn11-ml1 and Δmm2 mitochondrial morphology defects by Cdc14 overexpression reveals an uncharacterized connection between mitochondrial and cell cycle events. We propose a model (Fig. 7) in which the FEAR pathway, activating Cdc14, performs an essential function in regulating the mitochondrial morphology and function, ensuring proper segregation and maintenance of the mitochondrial network during cell cycle. Consistent with this model, most of the FEAR mutants exhibit important mitochondrial genome instability (rho- or rho−, data not shown). Figure 7 also illustrates the double function of the Rpn11 protein. As a proteasomal subunit, Rpn11 is involved in Pds1 degradation and hence in the activation of the FEAR pathway, whereas as a free-standing protein associated to the outer mitochondrial membrane, it is involved in interaction with the mitochondre proteins.

Mmm1, Mdm10 and Mdm12 form the core of the mitochondre, an outer mitochondrial membrane complex essential for the mitochondrial movement on the actin cytoskeleton and mtDNA maintenance. Mmm2 was described as a mitochondrial outer membrane protein associated with the mitochondre, involved in the assembly of the mitochondre complex (Youngman et al., 2004; Dimmer et al., 2005) and required for normal mitochondrial morphology and inheritance. Deletion of this gene results in collapsed mitochondria. Recently, it was demonstrated that Mdm10, Mdm12, Mmm1 and Mmm2 form the ERMES complex and provide a molecular tether between endoplasmic reticulum and mitochondria (Kornmann et al., 2009). We demonstrate here that the action of Cdc14 on Mmm2 function is specific, as overexpression of CDC14 in the deleted strains of the other ERMES components does not restore a tubular mitochondrial morphology. On the contrary, the overexpression of CDC14 completely suppresses the multibud phenotype of the Δmdm10 strain, in agreement with the observations of L. Pon and colleagues (Garcia-Rodriguez et al., 2009).

The variety of functions associated with the proteins belonging to the mitochondre/tubulation apparatus and ERMES complex (mitochondreial movement, mtDNA stability, protein import, endoplasmic reticulum–mitochondreia tether for metabolite exchange) suggests that these proteins are essential for the cross-talk between the mitochondreia and the rest of the cell. A coordination of organelle movement with cell cycle progression should exist, as the mitochondreia and the endoplasmic reticulum are the first two essential organelles to enter the bud.

Recently, Liza Pon’s group has proposed the existence of a mitochondrial checkpoint that is activated when the mitochondreia do not migrate correctly into the bud, as in the absence of Mdm10 the release of Cdc14 from the nucleolus is delayed (García-Rodríguez et al., 2009). Nevertheless, a regulation pathway for this coordination is still elusive. Our results are consistent with the hypothesis that a cross-talk exists between mitochondrial biogenesis and exit from mitosis in yeast in which the Rpn11 proteasomal protein is involved.

Acknowledgements

We would like to thank Dr B. Westermann for the kind gift of the plasmid PYX-mitoGFP, Marlene Eoffinger for the GAR1-GFP plasmid, Elmar Schiebel for the CDC14-GFP plasmid, Patrizia Filetici for the TUB1-GFP plasmid and Alessandro Fatica for helpful discussion. This work was supported by MIUR 2003, University of Rome ‘La Sapienza’ and FIRB (code RBNE01KMT9) to L.F. and by FRM to L.H.

References


Mdm10, Mdm12, Mmm1 and Mmm2 form the ERMES complex and


**Supporting Information**

Additional Supporting Information may be found in the online version of this article:

**Fig. S1.** Western blot analysis of W303-Gal-CDC14, rpn11-m1-Gal-CDC14 and Δmmm2-Gal-CDC14 strains.

**Fig. S2.** Overexpression of Cdc14 in the ERMES deleted strains (Δmmm1-GAL-CDC14, Δmdm10-GAL-CDC14, Δmdm12-GAL-CDC14) does not restore the mitochondrial tubular network.

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