Mitochondrion 12 (2012) 370–380

Contents lists available at SciVerse ScienceDirect

Mitochondrion

journal homepage: www.elsevier.com/locate/mito

Glucose levels regulate the nucleo-mitochondrial distribution of Mig2

Alejandra Fernández-Cid [1], Alberto Riera [1], Pilar Herrero, Fernando Moreno *

Department of Biochemistry and Molecular Biology, University of Oviedo, 33006-Oviedo, Spain

ARTICLE INFO

Article history:
Received 21 June 2011
Received in revised form 16 January 2012
Accepted 3 February 2012
Available online 12 February 2012

Keywords:
Glucose repression
Glucose signaling
Mitochondrial morphology
Mig2
Ups1
Yeast

ABSTRACT

Mig2 has been described as a transcriptional factor that in the absence of Mig1 protein is required for glucose repression of the *SUC2* gene. Thus, until now, the main role assigned to Mig2 has been the functional redundancy to Mig1. In this study, we report that Mig2 has a double subcellular localization. As expected, in high-glucose conditions it is accumulated in the nucleus but in low-glucose conditions Mig2 has an unexpected mitochondrial localization and role in mitochondrial morphology. We describe that Mig2 physically interacts with the mitochondrial protein Ups1 in a glucose-dependent manner. We also show that *Δmig2* mutant cells exhibit a fragmented network of mitochondrial tubules, a phenotype similarly observed in cells lacking Fzo1 and Ups1. Furthermore, Mig2 acts antagonistically with respect to the fission-promoting components, because mitochondrial aggregation induced by *DNM1* deletion was rescued in the *Δdnm1Δmig2* double mutant. Thus, our studies have revealed an additional role for Mig2 as a novel factor required for the maintenance of fusion-competent mitochondria in *Saccharomyces cerevisiae* and strongly suggest that Mig2 could be involved in the cross talk between the nucleus and the mitochondria through Ups1 to regulate mitochondrial morphology in a glucose dependent manner.

1. Introduction

Mig2 was identified several years ago as a repressor that collaborates with Mig1 to cause glucose-induced repression of *SUC2* gene (Lutfiyya and Johnston, 1996). A genome wide expression profiling survey of the yeast genome revealed that all the genes regulated by Mig1 are also regulated by Mig2. Thus, it was postulated that Mig2 always works in conjunction with Mig1 (Lutfiyya et al., 1998). However, Mig1 appears to be the essential component, since its presence is necessary and sufficient to cause full repression of most genes even in the absence of Mig2. Although the mechanism by which Mig2 participates in the glucose repression of *SUC2* gene is not completely understood, it is difficult to believe that the sole role of Mig2 is to partially repress gene expression in the absence of Mig1. In this work we describe a new function for this protein; our results suggest that Mig2 is a mitochondrial fusion-promoting protein in *S. cerevisiae*.

The mitochondria of many cell types fuse and divide continuously in a highly regulated manner, such that their overall structure can change rapidly in response to different biological cues. The importance of mitochondrial dynamics in cell life is demonstrated by the fact that defects in organelle fusion and fission lead to a variety of diseases in humans. Thus, mutations in mitochondrial-fusion components are associated with neurodegenerative diseases (Cho et al., 2010). Similarly, abnormalities in mitochondrial division are associated with defects in embryonic and postnatal development (Wakabayashi et al., 2009).

The major components of the mitochondrial fusion and fission machineries have been evolutionarily conserved from yeast to humans. Due to this conservation and the accumulated knowledge on the genetics and physiology of *S. cerevisiae*, this yeast emerged as one of the prime model organisms to study mitochondrial dynamics (Scott and Youle, 2010). Like other membrane-fusion events, mitochondria are first tethered together before their outer and inner membrane bilayers mix. Three conserved GTPases, Fzo1, Ugo1 and Mgm1, have central roles in mitochondrial fusion (Cohen et al., 2011; Herlan et al., 2004; Hermann et al., 1998; Sesaki et al., 2003; Zick et al., 2009). Similar to mitochondrial fusion, mitochondrial fission requires a GTPase, called Dnm1 (Bleazard et al., 1999; Otsuga et al., 1998). Fusion and fission processes are regulated by several proteins and it seems likely that additional yet unknown factors are involved. Among them, Ups1, a conserved intermembrane space protein, was originally identified to affect the processing of the GTPase Mgm1, a central component on the mitochondrial fusion machinery (Herlan et al., 2004; Sesaki et al., 2003) and thereby mitochondrial shape (Sesaki et al., 2006). Ups1 was later found to be required to maintain normal cardiolipin levels (Osman et al., 2009; Tamura et al., 2009) a phospholipid critical for mitochondrial structure and integrity. Thus, Ups1 regulates fusion by both alternative topogenesis of Mgm1 and control of

Abbreviations: HA, haemagglutinin; Myc, c-Myc peptide epitope; G6PD, glucose-6-phosphate dehydrogenase; GFP, green fluorescent protein; RFP, red fluorescent protein; DAPI, 4′,6-Diamino-2-phenylindole; SD, synthetic dextrose; SGly, synthetic glycerol; YEPD, yeast extract/peptone/dextrose; YEPGly, yeast extract/peptone/glicerol.

* Corresponding author at: Departamento de Bioquímica y Biología Molecular, Universidad de Oviedo, Campus de "El Cristo", 33006-Oviedo, Spain. Tel.: +34 985 103 567; fax: +34 985 103 157.
E-mail address: fmoreno@uniovi.es (F. Moreno).
[1] Present address: MRC Clinical Sciences Centre, Imperial College Faculty of Medicine, London, UK.

1567-7249/$ – see front matter © 2012 Elsevier B.V. and Mitochondria Research Society. All rights reserved.
doi:10.1016/j.mito.2012.02.001

mitochondrial cardiolipin metabolism. Dnm1 function is mainly regulated by Num1, assuring mitochondrial inheritance during mitosis (Cerveny et al., 2007).

Here we describe Mig2 as a novel mitochondrial associated protein involved in maintaining the morphology of these organelles. We also describe the glucose-dependent interaction between Mig2 and Ups1 and discuss a role of Mig2 protein in mitochondrial fusion control.

2. Materials and methods

2.1. Strains, growing conditions and plasmids

The *S. cerevisiae* strains used throughout this study were derived from W303.1A (Wallis et al., 1989) and BY4742 (Brachmann et al., 1998) haploid wild type strains and are listed in Table 1. Strain FMY501 expressing Mig2–GFP was constructed by homologous recombination in W303.1A using a GFP-HIS3 tagging cassette obtained from pFA6a-GFP-HIS3 plasmid (Longtine et al., 1998). FMY503 strain was constructed by homologous recombination in an Ups1-myc-TRP1 strain using a GFP-HIS3 tagging cassette obtained from pFA6a-GFP-HIS3 plasmid. FMY505 strain was constructed by homologous recombination in a Δ*dnm1* strain using a MIG2–His3MX6 deletion cassette obtained from the pFA6a-HIS3MX6 plasmid (Longtine et al., 1998). FMY506 strain was constructed by homologous recombination in a Δ*fzo1* strain using a MIG2–His3MX6 deletion cassette obtained from pFA6a-HIS3MX6 plasmid. FMY510 strain expressing Mig2–GFP was constructed by homologous recombination in a Δ*ups1* strain using a GFP-HIS3 tagging cassette obtained from pFA6a-GFP-HIS3 plasmid. FMY512 strain was constructed by homologous recombination in a Δ*mig2* strain using a UPS1-His3MX6 deletion cassette obtained from the pFA6a-HIS3MX6 plasmid. FMY534 strain was constructed by homologous recombination in a TetR–GFP strain using a Myc-TRP1 tagging cassette obtained from pFA6a-13Myc-TRP1 plasmid.

Escherichia coli DH5α (*F Ø80dlacZ ΔM15 recA1 endA1 gyrA96 thi-1 hsdR17(r_k-r_k-) supE44 relA1 deoRΔ 99U169*) was the host bacterial strain used for plasmid construction and amplification.

Yeast cells were grown in the following media: YEPD, high glucose (2% glucose, 2% peptone, and 1% yeast extract); YEPGly, low glucose (0.05% glucose, 3% glycerol, 2% peptone, and 1% yeast extract) and synthetic selective media containing the appropriate carbon source (2% glucose (SD, high glucose) or 3% glycerol and 0.05% glucose (SGly, low glucose) and 0.69% yeast nitrogen base without amino acids). Amino acids were added at a final concentration of 20–150 μg/ml. Solid media contained 2% agar in addition to the components described above.

pRS316-Su9-RFP a *CEN-URA3* plasmid expressing RFP fused to the presequence of subunit 9 of the F_o-ATPase of *Neurospora crassa* under control of the *ADH1* promoter was a gift from P. Sanz.

2.2. Fluorescence microscopy

Wild-type (Mig2–GFP) strain, Δ*mig2*, Δ*ups1*, Δ*dnm1*, Δ*fzo1*, Δ*dnm1*Δ*mig2* and Δ*fzo1*Δ*mig2* mutants were transformed with the centromeric plasmid pRS316-Su9-RFP and grown until early-logarithmic phase (OD_{600nm} of less than 0.8) in synthetic high-glucose selective medium (SD). Then, half of the culture was shifted to synthetic low-glucose selective medium (SGly) for 5 min. 25 μl of cells were loaded onto poly L-lysine-coated slides, and the remaining suspension was immediately withdrawn by aspiration. One μl of DAPI (2.5 μg/ml in 80% glycerol) was added, and a cover slide was placed over the samples. GFP, RFP and DAPI localization in live cultures was analyzed by direct fluorescence using a Leica DM5000B microscope. To avoid fluorescent signal in the non-linear range, cells overexpressing GFP- and RFP-tagged proteins were excluded from the analysis. Protein localization was estimated by visual inspection of the images. At least 100 cells were scored per experiment in three or more experiments. Fluorescence distribution was scored according to the following rule: N, denotes a nuclear fluorescence signal and M, mitochondrial fluorescence signal. Mitochondrial morphology was classified in four categories: wild type-like, fragmented, aggregated and others. Means and standard deviations are shown for at least three independent experiments. Representative images of the obtained results are shown. Images were processed using Adobe Photoshop CS.

2.3. Preparation of crude protein extracts

Yeast protein extracts were prepared as follows: yeasts were grown in 10 to 20 ml of synthetic glucose medium (SD; H-Glc) at 28 °C to an A_{600nm} of 0.8–1.0. Then, half of the culture was shifted to synthetic low-glucose medium (SGly, L-Glc) for 1 h. Cells were collected, washed twice with 1 ml distilled water and suspended in 200 μl PBS (pH 7.5) buffer containing Roche Protease Inhibitor plus 1 mM DTT and 0.1% Triton X100. Glass beads were added to the tubes and cells were broken using a FastPrep (Thermo Electron Co.) apparatus (two pulses of thirty seconds). Then, 200 μl of the same buffer were added to the suspension. Supernatant was recovered after 30 min of centrifugation at 4 °C (19,000 *g*). Supernatant was used as crude protein extract.

2.4. Isolation of mitochondria and nuclei

Cells were grown in 400 ml high-glucose YEPD at 28 °C to an $OD_{600\ nm}$ of 0.8. Then, half of the culture was shifted to low-glucose medium (YEPGly) for 1 h. Mitochondria were obtained as described previously (Daum et al., 1982) from half of the high and low glucose cultures. Nuclei were obtained from the remaining cultures as described previously (Randez-Gil et al., 1998).

Table 1
Saccharomyces cerevisiae strains used in this study.

Name	Relevant genotype	Source/Ref.
W303.1A	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1*	(Wallis et al., 1989)
BY4742	*MATα his3Δ 1 leu2Δ 0 met15Δ 0 ura3Δ 0*	(Brachmann et al., 1998)
FMY501	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 MIG2::GFP*	This work
FMY503	*MATa his3 leu2 lys2 trp1 ura3 UPS1-myc-TRP MIG2::GFP*	This work
FMY505	*MATα his3Δ 1 leu2Δ 0 met15Δ 0 ura3Δ 0 dnm1::kanMX4 mig2::HIS3*	This work
FMY506	*MATα his3Δ 1 leu2Δ 0 met15Δ 0 ura3Δ 0 fzo1::kanMX4 mig2::HIS3*	This work
FMY510	*MATa his3 leu2 lys2 trp1 ura3 ups1::kanMX4 MIG2::GFP*	This work
FMY512	*MATα his3Δ 1 leu2Δ 0 met15Δ 0 ura3Δ 0 mig2::kanMX4 ups1:: HIS3*	This work
FMY534	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 tetOx200::URA3 tetR–GFP::LEU2 UPS1-myc-TRP*	This work
TetR–GFP	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 tetOx200::URA3 tetR–GFP::LEU2*	(Michaelis et al., 1997)
YRJ2012	*MATa his3 leu2 lys2 trp1 ura3 UPS1-myc-TRP*	(Sesaki et al., 2006)
YRJ2011	*MATa his3 leu2 lys2 trp1 ura3 ups1::kanMX4*	(Sesaki et al., 2006)
Y01489	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 dnm1::kanMX4*	Euroscarf
Y03319	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 fzo1::kanMX4*	Euroscarf
Y14575	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 mig2::kanMX4*	Euroscarf