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The transcriptional activator *HAP4* is a high copy suppressor of an *oxa1* yeast mutation

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Abstract

Oxa1p is a key component of the machinery for the insertion of membrane proteins in mitochondria, and in the yeast *Saccharomyces cerevisiae*, the deletion of *OXA1* impairs the biogenesis of the three respiratory complexes of dual genetic origin. Oxa1p is formed from three domains located in the intermembrane space, the inner membrane and the mitochondrial matrix. We have isolated a high copy suppressor able to partially compensate for the respiratory deficiency caused by a large deletion of the matrix domain. We show that the suppressor gene corresponds to the nuclear transcriptional activator Hap4p which is known to regulate respiratory functions.

Keywords: Mitochondria; Respiratory complexes; *Saccharomyces cerevisiae*; Cytochrome oxidase; Membrane insertion

1. Introduction

The biogenesis of membrane oligomeric complexes of the mitochondrial respiratory chain is an intricate process that requires the co-assembly of mitochondrial and nuclear encoded subunits and the participation of nuclear-encoded factors that are not intrinsic components of the complex. Most of these proteins are conserved through evolution and several human neurodegenerative pathologies are due to respiratory complex assembly defects.

Oxa1p is functionally conserved from bacteria to eukaryotic organelles and is a key component of the insertion machinery of membrane subunits. In the yeast *Saccharomyces cerevisiae*, the deletion of *OXA1* impairs the biogenesis of the three respiratory complexes of dual genetic origin (complex III, IV and V) and the membrane subunits are rapidly degraded (Bonnefoy et al., 1994; Altamura et al., 1996; Lemaire et al., 2000). Moreover, Oxa1p seems essential for the translocation of the hydrophilic domain of the complex IV subunit, Cox2p (He and Fox, 1997; Hell et al., 1997; Herrmann and Bonnefoy, 2004). Oxa1p interacts with nascent mitochondrial polypeptides (Hell et al., 2001) and its C-terminal tail located in the mitochondrial matrix binds the mitochondrial ribosome. This interaction has been proposed to mediate the co-translational insertion of mitochondrially encoded subunits (Szyrach et al., 2003; Jia et al., 2003). Oxa1p show an N-out C-in topology (Herrmann et al., 1997) with three domains located in the intermembrane space, the inner membrane and the matrix. In order to clarify the precise role of these domains, we have constructed point and deletion mutants (Lemaire et al., 2004) and we have undertaken a systematic search for suppressors able to compensate for the respiratory deficiency due to various *oxa1* mutations.

We have already isolated two classes of extragenic suppressors able to compensate the respiratory deficiency of *oxa1* mutants. First, suppressor mutations located in the

Abbreviations: PCR, polymerase chain reaction; ORF, open reading frame; TM, trans-membrane domain.

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trans-membrane (TM) domains of two subunits of complex III, Cyt1p and Qcr9p, are able to compensate for the absence of Oxa1p (*Δoxa1*). We have proposed that these mutated TM domains of Cyt1p or Qcr9p could interact with the TM domains of other respiratory complex subunits leading to an insertion process independent of Oxa1p (Hamel et al., 1998; Saint-Georges et al., 2001). Second, the high copy suppressor gene, *OMS1*, encoding a methyl-transferase like protein, is specific for some *oxa1* alleles. The over-expression of *OMS1* increases the steady-state level of Oxa1p and thus facilitates the membrane insertion of respiratory subunits. This stabilization of Oxa1p could result from modifications of Oxa1p residues or of phospholipids (Lemaire et al., 2004). Thus, the suppression mechanisms are both acting at the assembly level but appear completely different in both cases.

In this paper, we describe the characterization of a new high copy suppressor of an *oxa1* mutation. We show that the suppressor gene corresponds to the transcriptional activator Hap4p which is known to regulate respiratory functions.

2. Materials and methods

2.1. Strains, media and genetic techniques

All strains were derived from the W303 nuclear background *MATα ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100*. The *oxa1* mutants were described in Lemaire et al. (2004). The *Δafg3* strain (*afg3::HIS3*) and the *Δhap4* (*hap4::G418*) strain are from A. Tzagoloff and B. Guiard. Yeast genetic methods and media used for *S. cerevisiae* have been described in Dujardin et al. (1980). Glucose or galactose were used as fermentable carbon sources and glycerol as a respiratory carbon source. Yeast cells were transformed by the lithium acetate procedure of Schiestl and Gietz (1989) for library screening.

2.2. High copy suppressor isolation

The *oxa1* mutant was transformed with a high copy library constructed by F. Lacroute into the *URA3* 2μ vector pFL44L. [Ura^+] clones were selected and replica-plated onto glycerol medium at 36 °C. Total yeast genomic DNA was extracted from fast or slow-growing co-segregating [Gly+] clones and used to transform *Escherichia coli* cells to recover the plasmids. Molecular analysis by restriction enzymes and sequencing identified the chromosomal fragments present in each plasmid.

2.3. Epitope tagging of Oxa1p

Oxa1p was tagged at its C-terminus with a six histidine epitope using the *Saccharomyces pombe HIS5* marker gene as described in Longtine et al. (1998). The PCR fragment was used to transform the starting strain to histidine prototrophy in order to fuse the tag to the end of the *OXA1* ORF in the yeast genome. Correct integration was confirmed by PCR amplification and sequencing. The strain CWOXA expressing the tagged protein is respiratory competent at 28 °C and 36 °C showing that the tagged protein is fully functional.

2.4. Isolation of mitochondria, Western blotting

Mitochondria were purified following the differential centrifugation procedures after digestion of cell walls by Zymoliase-100T (Kermorgant et al., 1997). The mitochondrial protein concentration was determined using the Bio-Rad assay. Mitochondrial proteins were separated on 12% acrylamide gels and blotted onto nitrocellulose membranes. Western blots were probed with various antibodies using the standard chemi-luminescence method.

3. Results and discussion

3.1. Over-expression of HAP4 compensates for the respiratory deficiency of oxa1-ΔL1-K332* mutant

In the search for genetic interactions involving the *OXA1* gene, we decided to search for high copy suppressor genes able to alleviate the respiratory defect of *oxa1* mutants. In *oxa1-ΔL1-K332**, the Oxa1p variant presents a large deletion of the first loop (L1) and a premature stop codon at position 332 in the C-terminal tail; both these domains protrude into the matrix. This mutant presents a tight respiratory deficiency associated to a strong defect in complex IV assembly and activity and a much weaker effect on complexes III and V (Lemaire et al., 2004). Three plasmids carrying overlapping fragments of chromosome XI (see Fig. 1A and B) were able to compensate for the respiratory deficiency of the mutation *oxa1-ΔL1-K332**. By subcloning the larger 7-kb insert of YEpSu1 (YEPSu1A and YEPSu1B), we demonstrated that the *HAP4* gene was responsible for the suppression. Surprisingly, the 4-kb fragment present in YEpSu8 and YEpSu22 exhibits a truncated *HAP4* gene encoding a 471-residue protein lacking the last 83 amino acids when compared to the full-length Hap4p. However, the over-expression of this truncated gene (*HAP4-S* allele) is as active in suppression as the complete *HAP4* gene.

Hap4p is responsible for the transcriptional activation capability of the Hap2/3/4/5 complex that positively regulates many of the genes involved in respiratory functions (Forsburg and Guarente, 1989). According to Bourgarel et al. (1999) and Stebbins and Triezenberg (2004), there is, in the C-terminal part of Hap4p, an activation domain that extends from the positions 450 to 471. In order to determine if the last 83 amino acids were required for the Hap4p activity, we have transformed the *Δhap4* strain with the plasmid carrying the truncated *HAP4-S* gene. As shown in Fig. 2, the *HAP4-S* gene is able to fully complement the respiratory defect of the *Δhap4* strain.