doi:10.1016/j.jmb.2007.09.044 J. Mol. Biol. (2007) 374, 506–516

JMB

Available online at www.sciencedirect.com

ScienceDirect

ELSEVIER

Knockdown of Human Oxa1l Impairs the Biogenesis of F_1F_o-ATP Synthase and NADH:Ubiquinone Oxidoreductase

Lukas Stiburek, Daniela Fornuskova, Laszlo Wenchich Martina Pejznochova, Hana Hansikova and Jiri Zeman*

Department of Pediatrics and Center of Applied Genomics 1st Faculty of Medicine Charles University, Prague 128 08, Czech Republic

Received 26 June 2007; received in revised form 17 September 2007; accepted 17 September 2007
Available online 20 September 2007

The Oxa1 protein is a founding member of the evolutionarily conserved Oxa1/Alb3/YidC protein family, which is involved in the biogenesis of membrane proteins in mitochondria, chloroplasts and bacteria. The predicted human homologue, Oxa1l, was originally identified by partial functional complementation of the respiratory growth defect of the yeast *oxa1* mutant. Here we demonstrate that both the endogenous human Oxa1l, with an apparent molecular mass of 42 kDa, and the Oxa1l-FLAG chimeric protein localize exclusively to mitochondria in HEK293 cells. Furthermore, human Oxa1l was found to be an integral membrane protein, and, using two-dimensional blue native/denaturing PAGE, the majority of the protein was identified as part of a 600–700 kDa complex. The stable short hairpin (sh) RNA-mediated knockdown of Oxa1l in HEK293 cells resulted in markedly decreased steady-state levels and ATP hydrolytic activity of the F_1F_o-ATP synthase and moderately reduced levels and activity of NADH:ubiquinone oxidoreductase (complex I). However, no significant accumulation of corresponding sub-complexes could be detected on blue native immunoblots. Intriguingly, the achieved depletion of Oxa1l protein did not adversely affect the assembly or activity of cytochrome *c* oxidase or the cytochrome bc_1 complex. Taken together, our results indicate that human Oxa1l represents a mitochondrial integral membrane protein required for the correct biogenesis of F_1F_o-ATP synthase and NADH:ubiquinone oxidoreductase.

Keywords: mitochondria; Oxa1l; ATP synthase; NADH:ubiquinone oxidoreductase; biogenesis

Edited by J. Karn

Introduction

The mitochondrial oxidative phosphorylation system (OXPHOS) is responsible for the vast majority of ATP produced in aerobic cells. It is composed of four respiratory chain complexes and the F_1F_o-ATP synthase (complex V) embedded within the inner membrane of the organelle. The biogenesis of OXPHOS is complicated by its sub-cellular location, dual genetic origin, the large number of constituent subunits and prosthetic groups, and the high hydrophobicity of some of the membrane subunits. Consequently, a number of specific gene products have evolved to accommodate such complex requirements. One of them is the Oxa1 protein, a founding member of the evolutionarily conserved Oxa1/Alb3/YidC protein family, which is involved in the biogenesis of membrane proteins in mitochondria, chloroplasts and bacteria.[1,2] The best characterized member of this family, *Saccharomyces cerevisiae* Oxa1, is an intrinsic protein of the inner mitochondrial membrane that mediates the insertion of mitochondrial translation products as well as of conservatively sorted nuclear gene products into the inner membrane from the mitochondrial matrix.[1,3,4] Although the yeast Oxa1 was shown to represent a rather

*Corresponding author. E-mail address: jzem@lf1.cuni.cz.

Abbreviations used: OXPHOS, oxidative phosphorylation system; CcO, cytochrome *c* oxidase; SDH, succinate:ubiquinone oxidoreductase; CS, citrate synthase; SDHA, 70 kDa flavoprotein subunit of SDH; PDH, pyruvate dehydrogenase; HEK293, human embryonic kidney 293; RNAi, RNA interference; shRNA, short hairpin RNA; shRNAmir, miR-30–based shRNA; FCCP, carbonyl cyanide *p*-(trifluoromethoxy) phenylhydrazone; GFP, green fluorescent protein; TMPD, *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine dihydrochloride.

general export machinery of the inner membrane, the co-translational membrane insertion of the mitochondrially encoded Cox2 precursor appears to exhibit the strictest dependency on its function.[5–7] The other substrates of Oxa1, including Oxa1 itself, can be inserted independently of its function, albeit with significantly reduced efficiencies.[8,9] Very recently, a novel post-translational role in the biogenesis of OXPHOS was demonstrated for yeast Oxa1. The protein was shown to stably interact in a post-translational manner with the ATP synthase subunit c, mediating its assembly into the ATP synthase complex.[10] Yeast *oxa1* cells are respiratory-deficient, with undetectable cytochrome *c* oxidase (CcO or complex IV) activity and markedly reduced levels of the F_1F_o-ATP synthase and cytochrome bc_1 complex (complex III).[3,4] *Schizosaccharomyces pombe* contains two distinct Oxa1 orthologues, both of which are able to complement the respiratory defect of yeast Oxa1-null cells. The double inactivation of these genes is lethal to this petite-negative yeast.[11] The depletion of Oxa1 in *Neurospora crassa* results in a slow-growth phenotype accompanied by reduced subunit levels of CcO and NADH:ubiquinone oxidoreductase (complex I). The *N. crassa*, Oxa1 was shown to form a 170–180 kDa homo-oligomeric complex, most likely containing four Oxa1 monomers.[12] Mitochondrial Oxa1 homologues possess a hydrophobic core domain composed of five transmembrane helices, and a C-terminal matrix domain that was shown in yeast to bind mitochondrial ribosome, thereby mediating co-translational membrane recruitment of nascent mitochondrial translation products.[13,14]

The predicted human Oxa1 orthologue, referred to as Oxa1l, shares 33% sequence identity with the corresponding yeast polypeptide. The human *OXA1L* cDNA was originally cloned by partial functional complementation of the respiratory growth defect of the yeast *oxa1-79* mutant.[15] It contains an open reading frame predicted to encode a protein of 435 amino acids. It was suggested that the ten exons of *OXA1L* might form an open reading frame able to encode a precursor protein of 495 amino acids,[16] and more recently the cDNA containing those additional 180 bp was cloned.[17] However, this extended version was shown to exhibit an even lower capacity to complement the respiratory growth defect of yeast *oxa1* cells than the original sequence.[18] The human *OXA1L* mRNA was found to be enriched in mitochondria-bound polysomes isolated from HeLa cells, and its 3′ untranslated region was shown to be functionally important when expressed in yeast cells.[18]

Here we address the role of human Oxa1l in the biogenesis of OXPHOS. We demonstrate here that human Oxa1l is a mitochondrial integral membrane protein that exists as part of a 600–700 kDa complex in mitochondria of human embryonic kidney 293 (HEK293) cells. We further show that the stable short hairpin RNA (shRNA)-mediated knockdown of human Oxa1l in HEK293 cells leads to markedly decreased protein levels and ATP hydrolytic activity of the F_1F_o-ATP synthase and moderately reduced levels and activity of NADH:ubiquinone oxidoreductase. Intriguingly, the activity and content of cytochrome *c* oxidase and the cytochrome bc_1 complex were unaffected or even increased in Oxa1l knockdown cells. Hence, these results indicate that human Oxa1l represents a mitochondrial integral membrane protein which is required for the correct biogenesis of the F_1F_o-ATP synthase and NADH:ubiquinone oxidoreductase.

Results

Human Oxa1l localizes to mitochondria in HEK293 cells

The predicted human Oxa1l precursor (Q15070) has a calculated molecular mass of 48.5 kDa and possesses a characteristic N-terminal mitochondrial targeting sequence. Both Mitopred and MitoProt II predict a significant score for location of the protein within mitochondria. To demonstrate the mitochondrial targeting of Oxa1l, a chimeric construct con-

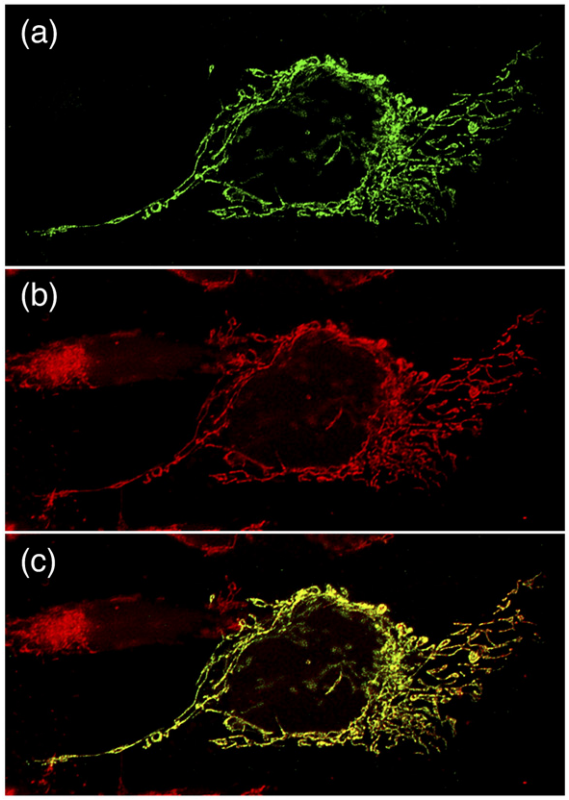

Figure 1. Overexpressed human Oxa1l–FLAG chimeric protein is targeted exclusively to mitochondria in HEK293 cells. (b) HEK293 cells were transiently transfected with the OXA1L–FLAG expression construct, stained with MitoTracker Red, and (a) subsequently labeled with a monoclonal M2 anti-FLAG antibody and with an anti-mouse Alexa Fluor 488 antibody. The fluorescence signal was recorded with a Nikon Eclipse TE2000 microscope, and deconvolved using the Huygens Professional Software (SVI). (c) Superimposition of (a) and (b) shows complete overlap of the two staining patterns.