Mitochondrion 24 (2015) 105–112

Contents lists available at ScienceDirect

Mitochondrion

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

Glutathione metabolism links *FOXRED1* to NADH:ubiquinone oxidoreductase (complex I) deficiency: A hypothesis

Bernard D. Lemire *

Department of Biochemistry, University of Alberta, Edmonton, Alberta T6G2H7, Canada

ARTICLE INFO

Article history:
Received 25 February 2015
Received in revised form 10 July 2015
Accepted 29 July 2015
Available online 30 July 2015

Keywords:
Leigh syndrome
NADH:ubiquinone oxidoreductase
Glutathione metabolism
Sarcosine oxidase
Nitric oxide
S nitrosylation

ABSTRACT

FOXRED1 mutations result in complex I (NADH:ubiquinone oxidoreductase) deficiencies and Leigh syndrome (subacute necrotizing encephalomyelopathy). FOXRED1 is a mitochondrial flavoprotein related to *N*-methyl amino acid dehydrogenases. How is FOXRED1 required for the biogenesis of complex I? I present a hypothesis that suggests FOXRED1 catalytic activity as a sarcosine oxidase protects the developing fetus from oxidative stress during pregnancy. Loss of FOXRED1, coupled with protein, choline and/or folate-deficient diets results in the depletion of glutathione, the dysregulation of nitric oxide metabolism and the peroxynitrite-mediated inactivation of complex I.

1. Introduction

The evolution of the mitochondrion has seen the extensive gain and loss of protein components. The mitochondrion and its now remnant genome evolved from a bacterial progenitor via an endosymbiotic event (Gray, 2012). The genome evolved though extensive gene loss, with the movement of genetic material to the nucleus. The human mitochondrial DNA (mtDNA) now encodes 13 protein subunits of the mitochondrial electron transport chain and ATP synthase. Addition and loss of mitochondrial proteins have occurred to different extents in different eukaryotic lineages.

One mitochondrial protein complex that has undergone extensive change since the original endosymbiotic event is the NADH:ubiquinone oxidoreductase (complex I) (Cardol, 2011; Gabaldón et al., 2005; Moparthi and Hägerhall, 2011). Mammalian complex I is composed of over 40 subunits, seven of which are still encoded in the mtDNA (Vinothkumar et al., 2014). In contrast, prokaryotic complex I normally consists of 11 to 14 highly conserved core subunits (Moparthi and Hägerhall, 2011). These core subunits harbor all the cofactors: an FMN and eight to nine iron-sulfur clusters (Efremov and Sazanov, 2011). Both the simpler prokaryotic and the more complex eukaryotic enzymes oxidize NADH, reduce a quinone acceptor and execute the vectorial movement of protons.

Diagnosis and treatment of mitochondrial diseases requires a better understanding of the biogenesis of the eukaryotic complex I. Additional complex I subunits added to the ancestral core enzyme are referred to as accessory or supernumerary subunits. For the most part, their roles remain poorly defined, but they have been suggested to be involved in enzyme assembly, stability or regulation (Brandt, 2006). The biogenesis of a fully assembled, functional complex I also depends on multiple assembly factors. Mutations affecting these assembly factors often result in complex I deficiencies and severe diseases, such as encephalomyopathy, cardiomyopathy and Leigh syndrome (Valsecchi et al., 2010).

FOXRED1 (FAD-dependent oxidoreductase) is a complex I-specific assembly factor (Fassone et al., 2010). Mutations in FOXRED1 result in Leigh syndrome (subacute, necrotizing encephalomyelopathy) and residual complex I activities of less than 10% normal levels (Calvo et al., 2010; Fassone et al., 2010). FOXRED1 has a mitochondrial targeting signal that is not removed upon import into mitochondria, but its role in complex I assembly has not been elucidated (Formosa et al., 2015).

Abbreviations: ABC, ATP-binding cassette; ADH3, alcohol dehydrogenase 3; ALDH, aldehyde dehydrogenase; CFTR, cystic fibrosis transmembrane conductance regulator; CPS1, carbamoyl phosphate synthase; DMGDH, dimethylglycine dehydrogenase; DHF, dihydrofolate; DMGO, dimethylglycine oxidase; dTMP, thymidylate; ETF, electron transfer flavoprotein; FGH, *S*-formylglutathione hydrolase; FGSH, *S*-formylglutathione; FOXRED1, FAD-dependent oxidoreductase; FTHFS, 10-formylTHF synthetase; GSH, glutathione; GSNO, *S*-nitrosoglutathione; GSNOR, GSNO reductase; GSSG, oxidized glutathione; HMGSH, *S*-hydroxymethylglutathione; iNOS, inducible nitric oxide synthase; IUGR, intrauterine growth restriction; MDH2, malate dehydrogenase; MSOX, monomeric sarcosine oxidase; mtDNA, mitochondrial DNA; MTHFD, methylene THF dehydrogenase; NO, nitric oxide; O_2^-, superoxide anion radical; ONOO-, peroxynitrite; PEMT, phosphatidylethanolamine-N-methyltransferase; PIPOX, peroxisomal sarcosine oxidase; ROS, reactive oxygen species; SARDH, sarcosine dehydrogenase; THF, tetrahydrofolate.

* Corresponding author.
E-mail address: bernard.lemire@ualberta.ca.

http://dx.doi.org/10.1016/j.mito.2015.07.009
1567-7249/

How is FOXRED1 participating in the biogenesis of complex I? Two cases of complex I deficiency due to mutations in *FOXRED1* have been reported (Calvo et al., 2010; Fassone et al., 2010). The genetics of both cases are consistent with a recessive mode of inheritance, suggesting loss-of-function mutations. Here, I present a hypothesis that links the loss of FOXRED1 catalytic activity to complex I biogenesis.

2. Hypothesis

A number of bioinformatic and structural observations are consistent with a catalytic function for FOXRED1.

1. Metazoans form the only large clade in which all members apparently have a FOXRED1. This suggests that complex I assembly is a function acquired by FOXRED1 during the evolution of metazoans. Highly conserved FOXRED1 orthologs are present in the genomes of many organisms without a complex I. An even larger number of organisms have a complex I but no FOXRED1 ortholog. These observations suggest that FOXRED1 is not essential for the biogenesis of complex I (except perhaps in metazoans) and are consistent with FOXRED1 having another function (Lemire, 2015a).
2. FOXRED1 is closely related to flavoprotein *N*-methyl amino acid dehydrogenases and oxidases, such as dimethylglycine dehydrogenase (DMGDH), sarcosine dehydrogenase (SARDH) and peroxisomal sarcosine oxidase (PIPOX). Nevertheless, FOXRED1 proteins have a distinct set of conserved residues that clearly distinguish them from these relatives. Multiple sequence alignments reveal highly conserved FOXRED1 residues buried near the FAD; these residues are also conserved in the FOXRED1 orthologs of organisms without a complex I (Lemire, 2015a). Investigation of FOXRED1 structure through molecular modeling suggests that the protein has a large substrate-binding pocket and may have an oxygen-binding site near its FAD (Lemire, 2015b).
3. In some proteobacteria, the *FOXRED1* gene is transcribed as part of an operon associated with the creatinine degradation pathway. FOXRED1 is co-expressed with the *N*-methylhydantoinase A and B subunits, which together hydrolyze *N*-methylhydantoin to *N*-carbamoylsarcosine. An amidase subsequently converts this product into sarcosine. FOXRED1, being closely related to SARDH, is likely capable of utilizing *N*-methyl amino acids, such as sarcosine, as a substrate.
4. Fungal FOXRED1 orthologs do not have amino-terminal mitochondrial targeting signals, suggesting they may be localized in the cytoplasm. A role for FOXRED1 in complex I biogenesis in these organisms has not been reported. However, a cytoplasmic localization is more consistent with a metabolic function in which a metabolite can be transported into mitochondria, than a chaperone-like function involving interactions with complex I subunits undergoing assembly (Lemire, 2015a).

Recently, FOXRED1 was found to be immunoprecipitable with complex I assembly intermediates (Andrews et al., 2013) or with several complex I subunits (Formosa et al., 2015). FOXRED1 association with a complex I assembly intermediates does not preclude a metabolic role. For example, the mitochondrial nucleoid contains the mtDNA and a large number of proteins, many involved in mtDNA binding and metabolism, but also many with roles unrelated to mtDNA maintenance (Bogenhagen, 2012). This latter type of protein includes metabolic enzymes, such as carbamoyl phosphate synthase (CPS1, urea cycle), malate dehydrogenase (MDH2, citric acid cycle) as well as chaperones and proteases (Bogenhagen et al., 2008; Wang and Bogenhagen, 2006). Indeed, even subunits of complex I can be identified in purified nucleoids. Thus, association with a complex does not inevitably imply that the association is functionally related. Alternatively, FOXRED1 may be a dual function protein.

In my hypothesis, summarized in Fig. 1, four steps link FOXRED1 to complex I.

1. FOXRED produces formaldehyde and glycine.
2. Glycine is important for glutathione metabolism.
3. Formaldehyde is important for folate-mediated one-carbon metabolism.
4. Glutathione depletion exacerbates oxidative stress and leads to a specific loss of complex I.

3. FOXRED1 produces glycine and formaldehyde

I will make the assumption that FOXRED1 functions as a sarcosine oxidase, converting sarcosine and O_2 to glycine, formaldehyde and H_2O_2 (Fig. 1). The architecture of the FOXRED1 active site is suggestive of sarcosine oxidase activity. One of the templates I used in my structural modeling of FOXRED1 was a monomeric sarcosine oxidase (MSOX; PDB: 2GF3) (Lemire, 2015b; Chen et al., 2006). FOXRED1, like MSOX, has a large active site cavity on the *re*-face of the FAD cofactor; the MSOX active site can accommodate not only sarcosine and dimethylglycine, but also pyrrole-2-carboxylate, the aromatic analog of proline (Wagner et al., 2000). Thus, a variety of *N*-methyl amino acids are possible substrates for FOXRED1; these include sarcosine and dimethylglycine. Also consistent with sarcosine oxidase activity is a large funnel-shaped pocket leading from the FOXRED1 surface to the FAD (Fig. S1) (Dundas et al., 2006), and a putative oxygen-binding site on the *si*-face of the FAD (Lemire, 2015b).

FOXRED1 sarcosine oxidase activity will release formaldehyde and glycine. Another modeling template I used was the *Arthrobacter globiformis* dimethylglycine oxidase (DMGO; PDB: 1PJ5) (Leys et al., 2003). DMGO has an N-terminal D-amino oxidase domain like FOXRED1, but it also has a folate-binding, C-terminal glycine cleavage T-protein domain. When DMGO is expressed in *E. coli*, very little formaldehyde is released. However, when its C-terminus is deleted, or when folate is absent, high levels of formaldehyde are generated (Tralau et al., 2009).

4. Glycine is needed for glutathione metabolism

4.1. The need for glycine

Glycine has numerous important structural and metabolic roles. Protein synthesis accounts for 80% of glycine needs in growing animals (Wang et al., 2013). Glycine accounts for 11.5% of total amino acids and 20% of amino acid nitrogen. Of particular interest for this hypothesis, glycine, cysteine and glutamate are joined to form glutathione, the most abundant intracellular, non-protein molecule and the major anti-oxidant in cells (Fig. 2, yellow area). Glycine is not considered a dietary essential amino acid but evidence suggests that endogenous synthesis is unable to meet metabolic demands under some conditions (Wang et al., 2013). One of these conditions is pregnancy (discussed below).

4.2. Choline is a source of glycine

Choline is an important source of glycine in mammals; it is converted to glycine via dimethylglycine and sarcosine via the actions of DMGDH and SARDH (Fig. 2, green area). In adult rats, almost half of ingested choline is metabolized to glycine and this fraction can increase to ~70% when choline ingestion is low (Wang et al., 2013). Choline is an essential nutrient for most people and choline deficiency is associated with liver and muscle dysfunction, oxidative stress, apoptosis and increased DNA damage (Zeisel, 2012). Many adult humans are choline-deficient and a number of studies report that only ~25% of Americans meet the recommended dietary intake of choline (Zeisel, 2012). Choline deficiency may be further aggravated by common